PB82-258799

Determination of Polynuclear Aromatic Hydrocarbons in Industrial and Municipal Wastewaters

Battelle Columbus Labs., OH

Prepared for

Environmental Monitoring and Support Lab.
Cincinnati, OH

Mar 82

U.S. Department of Commerce
National Technical Information Service
NTIS

PB82-258799

EPA-600/4-82-025
March 1982

DETERMINATION OF POLYNUCLEAR AROMATIC HYDROCARBONS IN INDUSTRIAL AND MUNICIPAL WASTEWATERS

by

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TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA-600/4-82-025	2. ORD Report	3. RECIPIENT'S ACCESSION NO. PB82 258799
4. TITLE AND SUBTITLE Determination of Polynuclear Aromatic Hydrocarbons in Industrial and Municipal Wastewaters		5. REPORT DATE March 1982
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Paul E. Strup		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Battelle - Columbus Laboratories 505 King Avenue Columbus, Ohio 43201		10. PROGRAM ELEMENT NO. 1BD612
		11. CONTRACT/GRANT NO. 68-03-2624
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Monitoring and Support Laboratory Office of Research and Development U.S. Environmental Protection Agency Cincinnati, Ohio 45268		13. TYPE OF REPORT AND PERIOD COVERED Final
		14. SPONSORING AGENCY CODE EPA/600/06
15. SUPPLEMENTARY NOTES		

16. ABSTRACT

A method for the determination of 16 polynuclear aromatic hydrocarbons (PAH) in wastewater was developed. This method, based on the use of high performance liquid chromatography with ultraviolet and fluorescence detection, is readily suited for detection of condensed ring aromatics in many types of aqueous samples. Aqueous sources included in this study are flyash wash water, municipal sewage, and industrial effluent samples.

Precision and accuracy of the method were estimated from the results of five wastewater samples spiked at levels between 0.1 and 250 ppb for the various PAH compounds. Recoveries were generally 85% or better from these wastewater sources.

Storage of several spiked wastewater samples for 0 and 7 days at various temperatures, pH and chlorine levels resulted in a matrix of recovery data for the various PAH species. This data indicated that the highest recovery for PAH in wastewater are obtained at a pH level near 7, in the absence of any chlorine with the analysis being completed as soon after collection as possible.

17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
18. DISTRIBUTION STATEMENT Release to public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 84
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory - Cincinnati conducts research to:

- Develop and evaluate techniques to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.

- Investigate methods for the concentration, recovery, and identification of viruses, bacteria, and other microbiological organisms in water. Conduct studies to determine the responses of aquatic organisms to water quality.

- Conduct an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.

Under provisions of the Clean Water Act, the Environmental Protection Agency is required to promulgate guidelines establishing test procedures for the analysis of pollutants. The Clean Water Act Amendments of 1977 emphasize the control of toxic pollutants and declare the 65 priority pollutants and classes of pollutants to be toxic under Section 307(a) of the Act. This report is one of a series that investigates the analytical behavior of selected priority pollutants and suggests a suitable test procedure for their measurement.

Robert L. Booth

Robert L. Booth, Acting Director
Environmental Monitoring and Support
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ABSTRACT

A method for the determination of 16 polynuclear aromatic hydrocarbons in wastewater was developed. This method, based on the use of high performance liquid chromatography with ultraviolet and fluorescence detection, is readily suited for detection of condensed ring aromatics in many types of aqueous samples. Aqueous sources included in this study are flyash wash water, municipal sewage, and industrial effluent samples.

Precision and accuracy of the method were estimated from the results of five wastewater samples spiked at levels between 0.1 and 250 ppb for the various compounds. Recoveries were generally 85% or better from these wastewater sources.

Storage of several spiked wastewater samples for 0 and 7 days at various temperatures, pH and chlorine levels resulted in a matrix of recovery data for the various species. These data indicated that the highest recovery for polynuclear aromatic hydrocarbons in wastewater are obtained at a pH level near 7, in the absence of any chlorine, with the analysis being completed as soon after collection as possible.

This report was submitted in fulfillment of Contract No. 68-03-2624 b. Battelle Columbus Laboratories under the sponsorship of the U.S. Environmental Protection Agency. This report covers a period from November 1, 1977, to March 1, 1979, and work was completed as of March 1, 1979.

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SECTION 1

INTRODUCTION

The objective of this program was to establish and verify procedures for the analysis of 16 priority pollutant polynuclear aromatic hydrocarbons (PAH) in complex aqueous media. Data obtained during this study will be used to aid in the selection of appropriate test procedures which will ultimately be used by the U.S. Environmental Protection Agency (EPA) in monitoring water and wastewater pollutants. These procedures should employ simple sample treatment as well as common laboratory instrumental and analytical approaches.

Sixteen polynuclear aromatics were studied in this program with regard to solvent stability, extraction efficiency, and preservation. These 16 PAH were: acenaphthene; benzo(ghi)perylene; fluorene; phenanthrene; dibenzo(ah)anthracene; ideno(1,2,3-cd)pyrene; acenaphthalene; anthracene, benzo(a)anthracene, benzo(a)pyrene; benzo(b)fluoranthene; benzo(k)fluoranthene; chrysene; fluoranthene; naphthalene; and pyrene. These compounds were combined into two groups for simultaneous study, in order to simplify initial analyses.

Further studies were then conducted to evaluate the extraction and analytical procedures that had been developed during this initial phase. These studies were performed using wastewaters obtained from various sources.

SECTION 2

OBJECTIVE

The objective of this study was to develop an optimized analytical method for the determination of 16 priority pollutant PAH in aqueous media and to validate the method on a variety of aqueous effluents.

SECTION 3

TECHNICAL APPROACH

The successful completion of this program involved the fulfillment of certain directives set forth in this contract by EPA. An extensive literature review was first conducted to evaluate the previous work in the area. Subsequent work was directed toward determination and then full evaluation of an appropriate measurement technique, which best satisfied the requirements for sensitivity and selectivity, as well as the considerations of sample cost, i.e. equipment, time, and training, which would be needed for the method. The stability of the PAH compounds in water miscible solvents and their instability in chlorinated and unchlorinated buffered water at different pHs and storage temperatures were studied over the prescribed time periods. Extraction efficiency of two organic solvents was also studied for the standard compounds. The remainder of the program involved the study of the sample preparation and cleanup steps which would be necessary to eliminate sample interferences. The complete method was then applied to several representative wastewater samples and an assessment was made of the precision and accuracy of the complete procedure.

SECTION 4

LITERATURE REVIEW

A literature review was conducted to establish the most attractive approach to the analysis of PAH in water. Although there is a large volume of literature on the subject, for the purposes of this report only a few of the most important references are presented. Discussions focus on the methodologies used for preservation, extraction, clean up, and determination of the PAH.

Preservation of PAH in water apparently has not been studied in detail in any single investigation. In one report the fate of PAH in water was studied, with special emphasis on the effect of UV light (1). In this study benz(a)anthracene and benzo(a)pyrene were used as model compounds since they are among the most reactive PAH. The conclusions reached by these authors include:

- The PAH are primarily adsorbed on particulate in natural water samples.
- The PAH are degraded to quinones when exposed to UV light above a certain threshold intensity.
- The degradation of PAH by UV light is not significantly affected by pH or ionic strength.

A second study (2) has evaluated the effect of chlorination on PAH stability. This study concluded that PAH can be degraded by added chlorine, especially at low pH and at elevated temperature. At low temperature and neutral or high pH the effect of chlorination is greatly reduced.

There are several methods reported for extraction of PAH from water including solvent extraction (1-7), polyurethane foams (8), and XAD-2 resin (9). In general, good recoveries have been obtained using all of these techniques, primarily because of the extremely hydrophobic nature of the PAH. Solvents used for extraction include benzene (1), isooctane (3), and methylene chloride (2,4,6,7). At least one report (7) concluded methylene chloride was the solvent of choice because of its good extraction properties and ease of work up.

There have been two methods reported for clean up of PAH extracts: a) solvent-solvent partition (4,7) and b) adsorption (column or thin layer) chromatography (3,4,8). One study (4) compared the recoveries using these

two techniques and found them to be equivalent. The solvent partitioning method involves 1) extraction of the PAH into isooctane, partitioning of PAH into dimethylsulfoxide (DMSO) or nitromethane, and 3) dilution of the extract with water and back extraction into isooctane. Adsorption chromatography has been performed using alumina (3), acetylated cellulose (8), and silica gel (4). Silica gel and alumina appear to be the best for this application since they are readily available in pure form and can be produced with uniform activity.

For the determination of PAH, gas chromatography/flame ionization detector (GC/FID) (1,2,8,9), gas chromatography/mass spectrometry (GC/MS) (3,4,6), thin layer chromatography (TLC) (7,10), and high performance liquid chromatography (HPLC) (11,12) have been employed. Thin layer chromatography does not appear to have sufficient resolution to separate all sixteen of the priority pollutant PAH and GC/MS is considered too expensive for the purposes of this study. Therefore, the candidate methods appear to be GC/FID and HPLC with a fluorescence detector. Although no direct comparisions of these two techniques for PAH analysis were found, it appears that HPLC with fluorescence detection is more sensitive than GC/FID whereas GC/FID may be somewhat more readily available. The largest unknown about both techniques is the degree of resolution which can be achieved and whether or not all sixteen PAH can be resolved. Separation of all sixteen PAH in a single chromatographic run has not been previously reported. A comparison of some of the characteristics of these two techniques is given below.

GC	HPLC
o Use of capillary column 20-30 meters will give 50-60K plates	o Packed columns (reverse or normal phase)
o Commercial coatings limited	o Commercial coating limited
o FID-universal detector	o UV or Fluorescence detector selective
o May require extensive sample clean up	o May require less extensive clean up
o Sensitivity advantage for lower MW compounds	o Greater sensitivity for selected compounds UV and/or fluorescence
o FID-linear response over wide range of concentrations	o UV-linear response over wide range of concentrations
o Possible one analysis for all PAH	o May require several fractionations for all PAH
o PAH identification by retention time only (with ready facility for GC/MS)	o PAH identification by retention time plus UV absorbance or fluorescence
o Automation easy	o Automation more difficult
o Investment low ($10K)	o Investment medium ($30K)
o Sample destroyed	o Sample not destroyed

SECTION 5

EXPERIMENTAL PROCEDURES

ANALYTICAL METHODS EVALUATION

The objective of this phase of the study was to ascertain which of the two chromatographic techniques (GC or HPLC) is most appropriate for the determination of the sixteen priority pollutant PAH.

Gas Chromatography (GC)

Glass capillary gas chromatography was investigated as an analytical technique for the 16 PAH. Various glass capillary columns were investigated using the following GC conditions:

Instrument - Hewlett-Packard 5730A
Detector - FID
Detection temperature - 300°C
Injector temperature - 300°C
Injection mode - 2μ L split 10:1
Column temperature - Group A - 2 minutes isothermal at 40°C
and then 40° - 260°C at 8°/minute.
Group B - 2 minutes isothermal at 80°C
and then 80° - 260°C at 8°/minute.

A spectra-Physics 4000 chromatographic data system was used to determine peak areas.

Detection limits for each compound were determined, based on a signal to noise ratio of 10:1.

High Performance Liquid Chromatography (HPLC)

Both normal and reversed phase forms of HPLC were investigated for the separation of PAH. For detection of the PAH both fluorescence (at various excitation and emission wavelength settings) and UV (fixed at 254 nm) were evaluated.

Stationary phases were packed in 4.6 mm I.D. x 25 cm stainless steel columns and were either purchased commercially or slurry packed using conventional techniques.

Solvents were filtered through .22 M Millipore filters and degassed by boiling for a few minutes prior to use on the HPLC. The chromatographic apparatus employed was as follows:

Solvent delivery system - Altex 420 microprocessor controlling dual Altex 100A pumps.
Injector - Rheodyne 7120 with a 20μ L loop.
Detectors - Fluorescence - Schoeffel FS970 UV-LDC UV-3 at 254 nm.
Data system - Hewlett-Packard Model 3385A.

SOLVENT STABILITY STUDIES

The purpose of this phase of the study was to determine which water miscible organic solvents are best for the preparation of standard solutions of the PAH for QA/QC purposes.

Solvent stability studies were conducted using DMSO and acetone as the solvents of choice. These solvents were selected because they are water miscible and dissolve readily PAH.

Four mL of acetone and DMSO solutions of PAH at a concentration of 20 ppm were sealed in glass ampules. The PAH were separated into two groups to facilitate the separation and quantitative analysis. These groups were: (A) naphthalene, anthracene, pyrene, chrysene, acenaphthene and dibenzo(ah)anthracene; and (B) phenanthrene, fluroene, benz(a)anthracene, fluoranthene, benz(a)pyrene, and acenaphthylene.

Benzo(k+b)fluoranthenes were not included in these studies due to an insufficient quantity of these standards. Because of supplier backlog, indeno(1,2,3-cd)pyrene and benzo(ghi)perylene were not incorporated into these initial groups and were analyzed independently.

The sealed ampules were kept in the dark, at room temperature for 30, 60 and 90 day time periods. Following chromatographic calibration, three ampules from each group were opened at the appropriate time interval. Analysis of PAH in acetone was conducted using capillary GC under the conditions described previously.

Because of solvent tailing, analysis of PAH In DMSO was performed by HPLC using the following conditions.

Column - 250 x 4.6 mm Spherisorb ODS 5μ particle diameter
Gradient - 50% acetonitrile in water to 100% in Acetonitrile in 50 minutes.
Flow - 1.0 mL/min
Solvent delivery system - Altex 420 microprocessor controlling dual altex 100A pumps.
Injector - Rheodyne 7120 with a 20 μL loop
Detector - UV-LDC UV-3 at 254 nm

Quantitation for both GC and HPLC was conducted by peak area integration using either a Spectra Physics 400 or Hewlett-Packard 8085A integrator. Calibration curves were prepared by appropriate dilution of a standard stock solution which were run at suitable sensitivity levels. Peak areas of interest were compared to the calibration curves and reported as percent recovery.

EXTRACTION STUDIES

The purpose of this phase of the study was to determine the extraction efficiencies of the various PAH under a variety of conditions, in order to select an optimized extraction protocol.

Extraction studies were conducted to evaluate the extraction efficiencies of methylene chloride and 15 percent methylene chloride in hexane for PAH in water at pHs 2, 7, and 10.

One mL of standard solution in acetone was added to 500 mL of appropriately buffered water to yield an approximate concentration of 40 ppb for each of the PAH in a 100 mL separatory funnel. Certified pH 7 and pH 10 buffers were purchased from VWR. The buffers used are as follows:

pH 2 - NaH_2PO_4 in phosphoric acid, 0.05 M Ionic Strength

pH 7 - sodium and potassium phosphate, 0.05 M Ionic Strength

pH 10 - sodium borate and sodium carbonate, 0.05 M Ionic Strength

PAH were extracted three times using 30 mL portions of the appropriate solvent. The extract was then dried with $MgSO_4$, filtered, and the $MgSO_4$ washed with an additional 30 mL of solvent. The resultant extract was reduced in volume to one mL using micro Kuderna-Danish evaporation. Extractions were done in triplicate for pH 2 and 10 and quadruplicity for pH 7.

Triplicate analysis on each of the above extracts was conducted using a capillary column GC equipped with a Hewlett-Packard 7671A automatic sampler.

Benzo(b)fluoranthene was not included in these studies due to an insufficient quantity of this standard.

PRESERVATION STUDIES

The purpose of this phase of the study was to examine the effects of sample storage under a variety of pH, temperature, and oxidant conditions in order to develop protocols for sampling and sample storage.

Preservation studies were conducted to evaluate the stability of PAH in water for selected conditions. These conditions were: pH 2, 7, and 10; chlorine 0 and 2 ppm; and temperature, ambient and 4°C. The three pH levels were achieved using the buffers described in the Extraction Section. The two ppm chlorine level was achieved by spiking the sample with 2 mL of a solution containing 1500 ppm of calcium hypochlorite

One mL of standard PAH solution in acetone was added to 500 mL distilled water, (to achieve a concentration of 40 ppb for each PAH) which had previously been buffered and/or chlorinated in accordance with the above matrix. Each matrix was performed in two groups as in the previous studies with the addition of benzo(k)fluoranthene and indeno-(1,2,3-cd)pyrene to Group A and benzo(ghi)perylene to Group B. All matrix series were stored in the dark for a period of 7 days: one series at ambient temperature, the other at 4°C. Each condition was run in duplicate.

Following the 7 day storage period, each solution was extracted with methylene chloride as described in the Extraction Studies section. The storage bottle was also rinsed with each 30 mL portion of methylene chloride prior to being used for extraction in the separatory funnel. Each extract was then dried, and reduced in volume as described above. Duplicate injections were made onto a capillary column GC equipped with a Hewlett-Packard 7671A automatic sampler. Quantitation was performed by peak area integration using a Spectra Physics 4000 integrator. Calibrations were performed every 24 hours with periodic calibration checks at four run intervals.

LC CLEAN UP STUDIES

The purpose of this phase of the study was to evaluate several clean up techniques in order to determine which technique gives best recoveries for the various PAH.

Recoveries of PAH from two LC clean up procedures were investigated. Each LC separation scheme was performed by applying 0.5 mL of a 200 ppm cyclohexane solution of representative PAH. In solution, the PAH chosen on the basis of representative ring number and relative elution volumes were: naphthalene, anthracene, fluoranthene, chrysene, and dibenz(a,h)-anthracene.

The alumina LC column was prepared by placing 10 g of alumina (3% H_2O w/w) in a 25 x 1 cm column, and wetting with 15 mL pentane. As the pentane reached the top of the alumina, 3/4" of Ha_2SO_4 was added. The column was washed with 20 mL of methylene chloride and then recondi-tioned with 20 mL of pentane. The PAH extract was then placed on the head of the column and eluted with 25 mL of pentane and 25 mL of methyl-ene chloride. The latter methylene chloride fraction contained the PAH. The LC scheme is presented in Figure 1.

The silica gel LC column was prepared by slurry packing 10 g of silica gel (Davidson Grade 923, 100-200 mesh) with methylene chloride

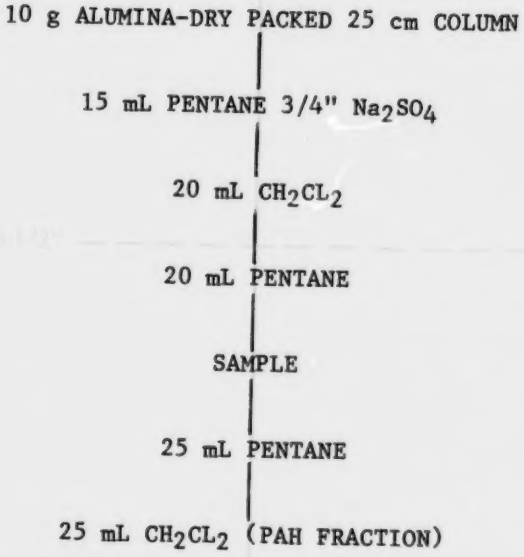

Figure 1. Alumina LC clean up scheme used for PAH.

into a 1 x 25 cm LC column. The column was washed with 40mL of pentane. The sample extract was then placed on the head of the silica gel and eluted with 25 mL of pentane and 25 mL of 40% methylene chloride in pentane. This letter fraction contained the PAH of interest. This LC scheme is presented in Figure 2. After allowing the first 25 mL of pentane to pass through the LC column, successive 10 mL fractions were collected and analyzed by HPLC using UV detection at 254 nm. No volume reduction was performed since the initial concentration of PAH was chosen to eliminate this step.

WASTEWATER STUDIES

The purpose of this phase of the study was to evaluate the analytical protocols, developed on the basis of information collected in earlier phases of this study, on actual wastewater samples and to make modifications as required.

Based on extraction efficiency studies, the following procedure was initially employed for the analysis of actual wastewater samples as supplied by EPA as well as samples collected by our labortories.

- Extract immediately three 1 liter aliquots of each wastewater using 3 x 60 mL methylene chloride.
- Spike six 1 liter aliquots of each wastewater with PAH compounds prior to extraction, with 3 aliquots being stored for 7 days at 4°C.
- Pour extract through drying column containing sodium sulfate.
- Reduce volume of extract to 1.0 mL using Kuderna-Danish concentrator apparatatus.
- Exchange solvent with cyclohexane and perform silica gel clean up technique (Figure 2).
- Concentrate the PAH fraction to 2-3 mL using Kuderna-Danish concentrator apparatus.
- Exchange solvent with acetonitrile and adjust the extract volume to 1.0 mL.
- Analyze by injecting 5 µL into the HPLC equipped with an HC-ODS column and fluorescence detector at λex 280 and λem 389.

Quantitation was performed by comparing the peak areas for the individual PAH species to those of a standard made up to the concentration representing 100% recovery.

Following discussions with EPA personnel, the extraction procedure was modified so that all concentration steps were performed using a

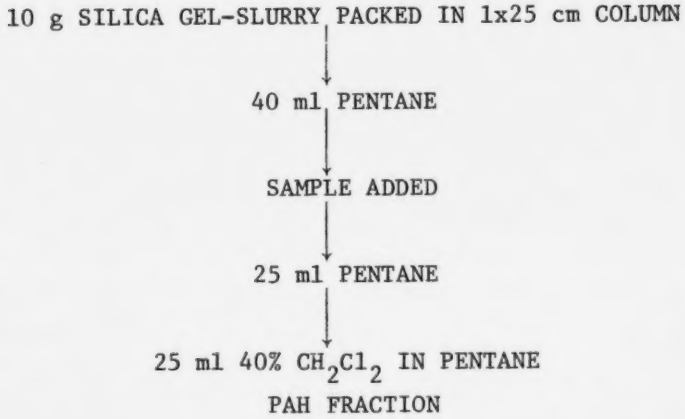
10 g SILICA GEL-SLURRY PACKED IN 1x25 cm COLUMN
40 ml PENTANE
SAMPLE ADDED
25 ml PENTANE
25 ml 40% CH_2Cl_2 IN PENTANE
PAH FRACTION

Figure 2. Silica Gel LC clean-up scheme used for PAH.

hot water bath rather than a tube heater. Also a 254 nm UV detector was coupled to the fluorescence detector to achieve lower detection limits for naphthalene and acenaphthylene. The revised procedures are described in detail in Appendix A.

The following water samples were selected for analysis:

- A plastics manufacturing industry (Samples supplied by EPA-Source Unknown).
- Raw municipal sewage water (Columbus, Ohio).
- Treated municipal sewage water prior to chlorination (Columbus, Ohio).
- Flyash Wash from coal fired power plant (Columbus, Ohio).
- Flyash settling pond prior to runoff (Columbus, Ohio).

All wastewater samples were collected by our laboratories except the plastics manufacturing industry wastewater which was supplied by EPA.

Extractions were conducted immediately on three 1 liter aliquots of each wastewater as received. In addition, six 1 liter aliquots were spiked with PAH at a level at least 5 times background prior to extraction, 3 aliquots being stored for 7 days at 4°C.

SECTION 6

RESULTS AND DISCUSSION

ANALYTICAL METHODS EVALUATION

Gas Chromatography

Experimental GC conditions were based on our considerable experience with analysis of PAH utilizing capillary columns. Although PAH separation in this study was achieved using an SE-30 capillary column, equal resolution has been achieved using capillary columns coated with SP-2100, OV-101, and SE-54. Examples of these separations are shown in Figures 3 and 4, with retention times given in Table 1.

The conditions used for GC analysis are as follows:

30 meter SE-30 glass capillary
FID detector - 300°C
Injector - 200°C
2 L split injection 10:1 at 40°C Group A: 80°C Group B
Isothermal for 2 min then programed 8°C/min to 260°C

Instrument detection limits for the various PAH, using capillary GC, are listed in Table 2. Due to the rather poor peak shape, and resulting poor detection limits and resolution, for the high molecular weight PAH (e.g. indeno-pyrene) capillary column GC was not pursued further as an analytical approach. However, use of specially coated capillary columns could reduce these problems and for the lower molecular PNA (e.g. naphthalene, anthracene, etc.) GC is probably a very good approach.

High Performance Liquid Chromatography

Normal and reverse phase HPLC were investigated for the separation of the PAH of interest. There is much in the literature describing the separation of a limited number of PAH using reverse phase HPLC (11,12); however, no references could be located on the separation of all 16 PAH of interest using a single stationary phase.

Initial HPLC reverse phase separations were conducted using a 250 x 4.6 mm Spherisorb ODS 5μ column using a 75-100% MeOH in water gradient elution in 30 min. This gradient gave acceptable resolution for PAH in Group A but failed to seprate fluorene and phenanthrene in Group B. Although using a longer gradient elution time (i.e. 40 min)

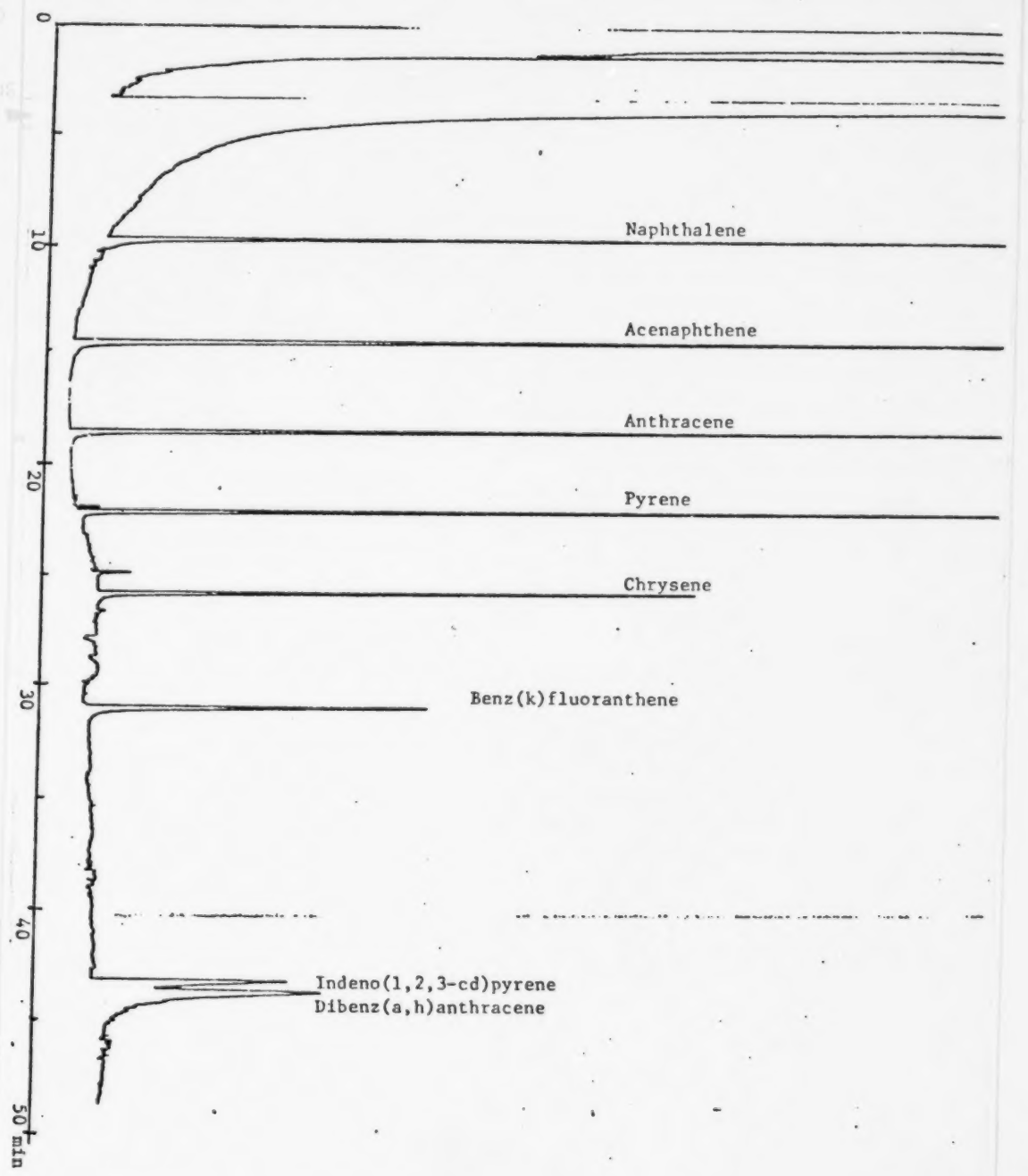

Figure 3. GC capillary column separation of Group A PAH.

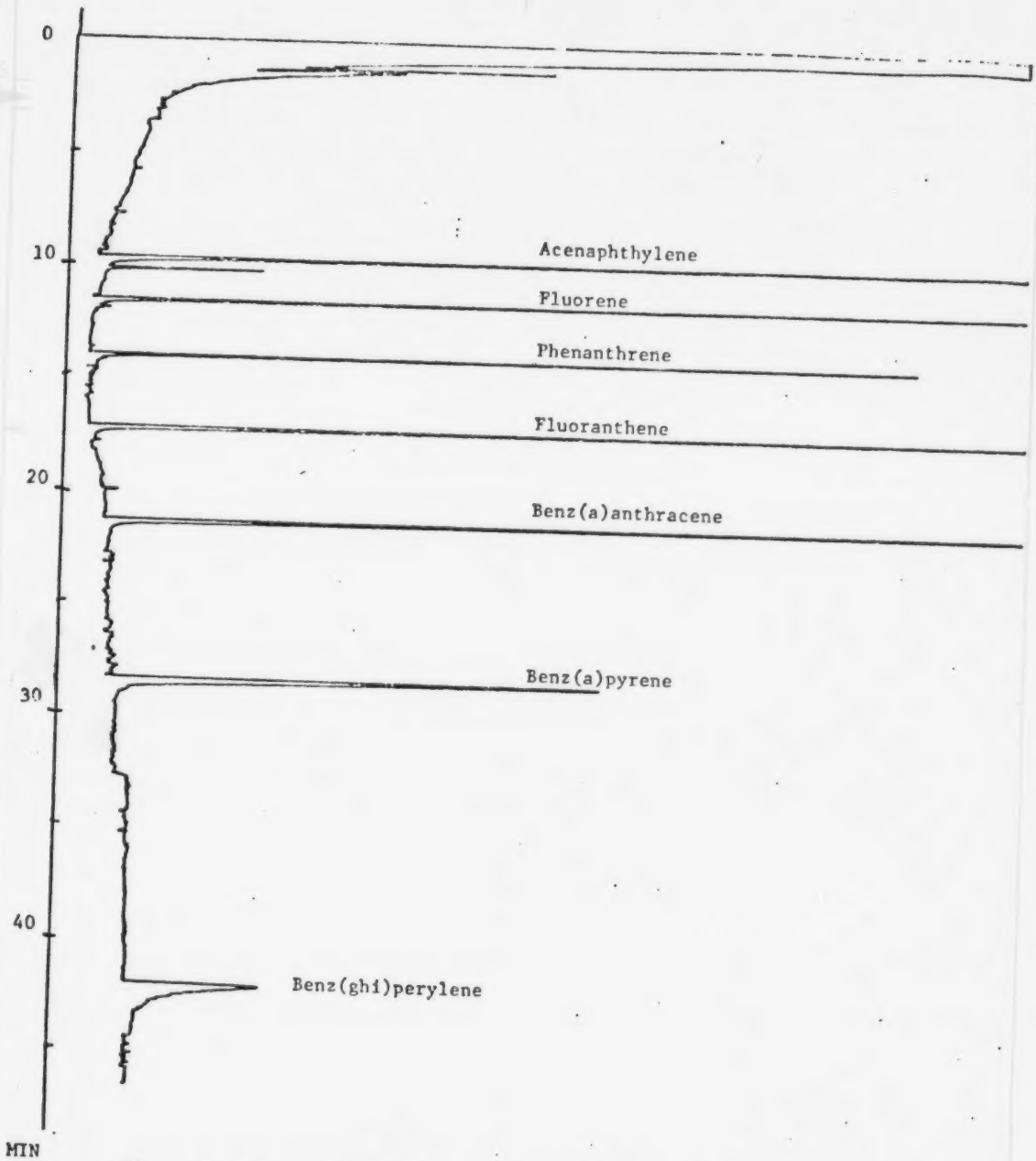

Figure 4. GC capillary column separation of Group B PAH.

TABLE 1. GC CAPILLARY COLUMN RETENTION TIMES FOR PAH ON 30 METER SE-30

Group A		Group B	
Compound	RT (min)*	Compound	RT (min)*
Napthalene	9.53	Acenaphthylene	9.65
Acenaphthene	14.30	Fluorene	11.51
Anthracene	20.70	Phenanthrene	13.95
Pyrene	21.98	Fluoranthene	17.21
Cyrysene	25.81	Benz(a)anthracene	21.40
Benz(a)fluoranthene	30.93	Benz(a)pyrene	28.37
Indeno(1,2,3-cd)pyrene	43.49	Benz(ghi)perylene	42.10
Dibenz(a,h)anthracene	43.84		

* Elution conditions for Group A and B were different so that retention times cannot be directly compared.

TABLE 2. PAH DETECTION LIMITS

Compound	Capillary GC FID 10:1 split (ng)	UV 254 (ng)	Fluorescence λex 280, λem >389 (ng)
Naphthalene	1.0	2.5	20.0
Acenaphthylene	1.5	5.0	100.0
Acenaphthene	.5	3.0	4.0
Fluorene	2.0	0.5	2.0
Phenanthrene	2.0	0.25	1.2
Anthracene	2.0	0.10	1.5
Fluoranthene	1.0	0.50	0.05
Pyrene	2.0	0.10	0.05
Benz(a)anthracene	2.0	0.20	0.04
Chrysene	4.0	0.20	0.5
Benz(b)fluoranthene	---	1.0	0.04
Benz(k)fluoranthene	6.0	0.30	0.04
Benz(a)pyrene	3.0	0.25	0.04
Dibenz(a,h)anthracene	10.0	1.0	0.08
Benz(ghi)perylene	---	0.75	0.2
Indeno(1,2,3-cd)pyrene	10.0	0.30	0.1

Minimum detectable quantity in nanograms injected at a signal to noise ratio of 10:1

achieved this separation, acetonitrile in water proved to be more selective and gave a flatter base line.

Moderate success on separation of all 16 PAH was achieved using a gradient elution of 50% acetonitrile to 100% acetonitrile in 50 minutes. An example of this separation is shown in Figure 5. However, the important isomer pair, benz(a)anthracene/chrysene as well as dibenz(a,h)anthracene/benz(ghi)perylene, are not completely resolved. This latter pair could be resolved using an isocratic step program, but these conditions still left benz(a)anthracene and chrysene unresolved.

Several normal phases for the separation of PAH were investigated. These phases included Lichrosorb Si60, alumina, and Lichrosorb NH_2. Hexane was the mobile phase in all cases. The results of these studies indicated that there were not enough efficiency or selectivity to separate all 16 PAH.

Further investigation of various reverse phase columns indicated that the separatin of all 16 PHA could be achieved using a Perkin-Elmer 10μ , 0.26 x 25 cm HC-ODS column. Figure 6 shows the separation of all 16 consent decree PAH using a single staionary phase. The conditions used for this separation were 50% acetonitrile in water isocratic for 5 minutes followed by a gradient elution from 40-100% acetonitrile in 25 minutes at a flow rate of 0.5 mL/min. Fluorescence detection was used with excitation at λex 280 nm and a cut off filter of λem >389 nm. PAH retention times using the Spherisorb and HC-ODS columns are given in Table 3.

Sensitivity

Since all 16 PAH compounds can be resolved using the Perkin-Elmer HC-ODS reverse phase column, it is possible to optimize a fluorescence or UV detector for the best signal to noise ratio for each compound. Fluorescence at λex 280 nm, λem >389 nm gives good sensitivity for most of the larger ring PAH compounds but does not have good sensitivity for the smaller ring compounds like naphthalene and acenaphthylene.

Although these studies were carried out using fluorescence at λex 280 nm, λem >389 nm, samples were spiked at levels approximately 25 times the minimum detection limit (i.e. 25 x the detection limit in ng on column assuming a 1000:1 concentration factor and 5 microliter injection volume), making the choice of wavelength setting of little consequence. However, for increased sensitivity for the smaller ring PAH compounds a UV detector at 254 nm can be placed in series with the fluorescence detector. Sensitivities obtained using these wavelengths are listed in Table 2. Detector response was found to be linear to 25 x these sensitivities in terms of nanograms on column which covered the range in which subsequent samples were spiked.

Details of the recommended analytical procedure for the analysis of PAH in industrial wastewater, based on the results of these studies is given in Appendix A.

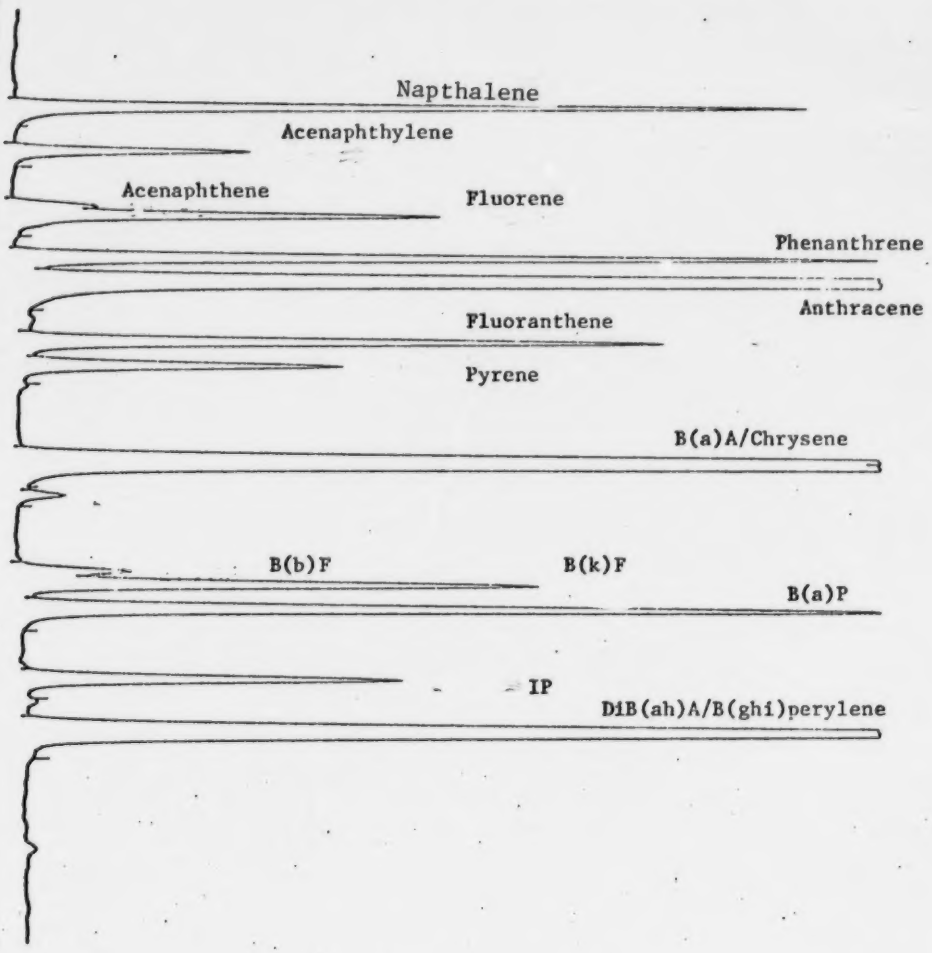

Figure 5. Chromatogram of HPLC separation of 16 PNA standards.

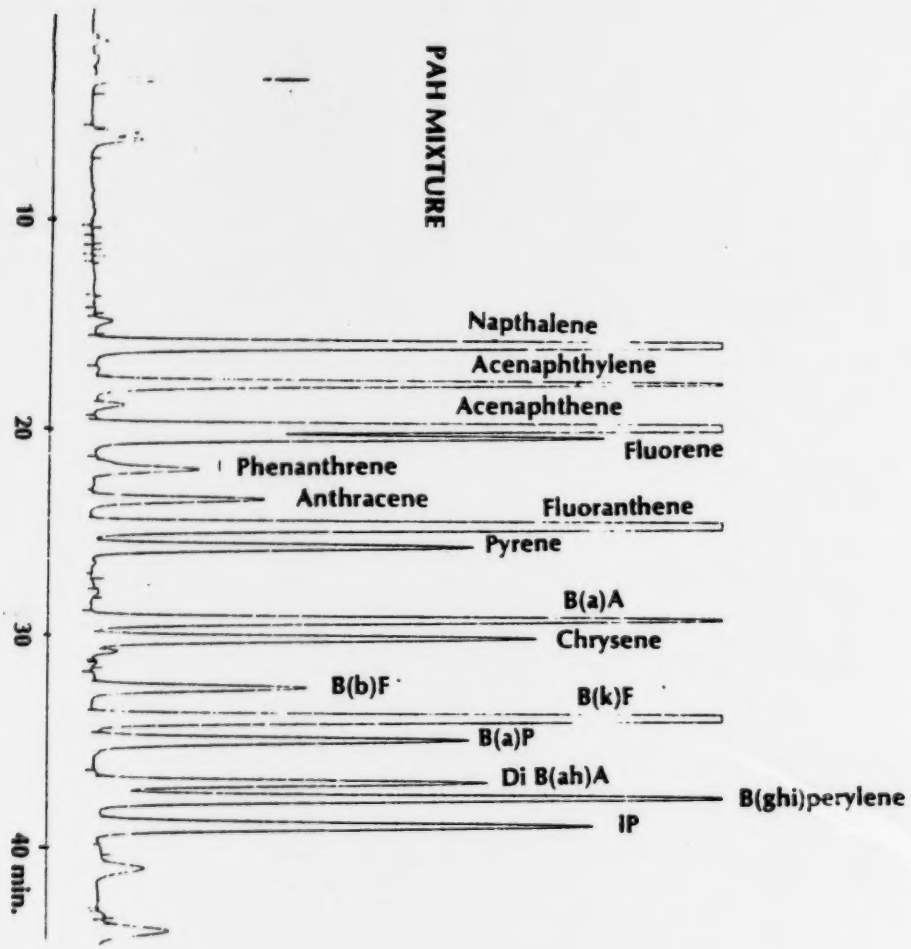

Figure 6. HPLC separation of 16 PAH standards on HC-ODS.

TABLE 3. HPLC RETENTION TIMES FOR PAH ON REVERSE PHASE COLUMNS

	Column: Spherisorb ODS 4.6 x 250 nm Gradient: Acetonitrile/H_2O 50%-100% 50 min Flow: 1 ml/min	Column: Perkin-Elmer HC-ODS 2.6 x 250 mm Gradient: Acentonitrile/H_2O in 40%-5 min 40%-100%-25 min Flow: 0.5 ml/min
Compound	Retention time (Minutes)	Retention time (Minutes)
Naphthalene	11.45	16.17
Acenaphthylene	13.00	18.10
Acenaphthene	14.91	20.14
Fluorene	15.21	20.89
Phenanthrene	16.66	22.32
Anthracene	17.46	23.78
Fluoranthene	19.58	25.00
Pyrene	20.41	25.94
Benz(a)anthracene	23.63	29.26
Chrysene	23.63	30.14
Benz(b)fluoranthene	27.56	32.44
Benz(k)fluoranthene	28.01	33.91
Benz(a)pyrene	28.85	34.95
Dibenz(a,h)anthracene	33.06	37.06
Benz(ghi)perylene	33.06	37.82
Indeno(1,2,3-cd)pyrene	31.30	39.21

Variance of Detector Response

The variance of the fluorescence detector response was checked to determine if there were any affect on detector response (e.g. dissolved oxygen in the HPLC mobile phases) over a period of time.

Spectral grade acetonitrile and high purity water were passed through a .22 μM Millipore filter prior to boiling each mobile phase for a period of 5 minutes. After allowing them to cool, each mobile phase was poured through one gradient elution with no injection. Following equilibration, a normal separation was performed by injecting a PAH solution. A further PAH standard separation was performed at the end of the day. These standard analyses continued for a period of three days which is approximately the duration before the mobile phases need to be replenished. The peak areas for each PAH component were averaged for the six runs and the standard deviation from the average determined. The results which are listed in Table 4 indicate that over a period of three days, there is only a slight variance in the detector response; and since recoveries are calculated using daily calibrations, these variances would not substantially affect analytical results.

Variance in Retention Times

Absolute retention times for individual PAH compounds was found to vary somewhat day to day. Although a microprocessor was used to control the entire gradient and reconditioning of the column prior to injection, these variances nevertheless occurred.

The initial gradient concentration of 40% acetonitrile in water was held for a period of 10 minutes to obtain column equilibrium prior to injection. Holding this concentration for 30 minutes before injection yielded no decrease in the variance. Thus, for shorter total analysis time a 10 minute reconditioning time was used prior to sample injection.

The variances which were obtained on the six PAH standard solutions over a period of three days are listed in Table 5.

SOLVENT STABILITY STUDIES

Results of the solvent stability studies are shown in Table 6. An ANOVA analysis of these data indicates that acetone and DMSO are virtually equally suitable solvents for the PAH studied for a time period of 90 days. ANOVA analysis of the data indicated a significant difference favoring acetone over DMSO for fluoranthene; however, examination

TABLE 4. VARIATION IN FLUORESCENCE DETECTOR RESPONSE

Compound	Relative percent standard deviation λex 280/λem >389 % (n = 6)*
Naphthalene	±3
Acenaphthylene	±3
Acenaphthene	±3
Fluorene	±3
Phenanthrene	±2
Anthracene	±1
Fluoranthene	±2
Pyrene	±2
Benz(a)anthracene	±1
Chrysene	±1
Benz(b)fluoranthene	±1
Benz(k)fluoranthene	±3
Benz(a)pyrene	±7
Dibenz(a,h)anthracene	±2
Benz(ghi)perylene	±5
Indeno(1,2,3-cd)pyrene	±2

* Over a 3 day period.

TABLE 5. VARIANCE OF RETENTION TIME

Compound	Average retention time Time (min)	Standard deviation (n = 6) Time (min)
Naphthalene	16.17	±0.31
Acenaphthylene	18.10	±0.27
Acenaphthene	20.14	±0.50
Fluorene	20.89	±0.24
Phenanthrene	22.32	±0.24
Anthracene	23.78	±0.23
Fluoranthene	25.00	±0.24
Pyrene	25.94	±0.24
Benz(a)anthracene	29.26	±0.23
Chrysene	30.14	±0.22
Benz(b)fluoranthene	32.44	±0.20
Benz(k)fluoranthene	33.91	±0.19
Benz(a)pyrene	34.95	±0.20
Dibenz(a,h)anthracene	37.06	±0.18
Benz(ghi)perylene	37.82	±0.19
Indeno(1,2,3-cd)pyrene	39.21	±0.18

TABLE 6. SOLVENT STABILITY STUDIES

Compound	DMSO					Acetone				
	0 day	30 day	60 day	90 day	Average	0 day	30 day	60 day	90 day	Average
Naphthalene	100±5*	99±5	101±2	95±2	99±4	100±1	96±9	99±2	104±1	100±4
Acenaphthene	98±6	97±7	104±5	100±3	100±5	100±0.6	95±6	98±2	102±3	99±4
Anthracene	100±0.6	97±0.6	99±1	97±0.6	98±2	100±2	98±5	96±2	100±5	98±4
Pyrene	100±0.6	99±2	99±0.6	100±2	100±1	99±2	99±5	100±2	98±3	99±3
Chrysene	100±0	97±0.6	99±0	99±2	99±1	100±4	99±6	100±2	104±0.6	101±4
Indeno(1,2,3-cd)pyrene	101±3	99±2	97±0.6	99±3	99±2	99±2	99±2	97±6	98±5	98±4
Dibenz(a,h)anthracene	101±2	99±4	101±3	100±3	100±2	98±3	95±6	95±9	98±7	97±6
Acenaphthylene	99±2	101±3	98±2	102±2	100±3	100±4	100±4	103±2	104±2	102±3
Fluorene	100±1	101±2	101±2	102±1	101±2	100±7	102±2	104±1	105±3	103±4
Phenanthrene	100±1	100±3	98±2	102±1	100±2	100±3	103±4	102±1	101±3	102±3
Fluoranthene	100±1	98±3	95±2	100±1	98±3	100±3	101±2	100±0.6	101±4	101±2
Benz(a)anthracene	100±0	100±3	96±2	102±0.6	100±3	100±4	103±3	98±5	95±2	99±5
Benz(a)pyrene	100±1	103±5	95±2	103±3	100±4	100±5	89±10	102±2	97±8	97±8
Benz(ghi)perylene	100±4	101±3	95±2	101±3	99±3	100±3	99±2	100±3	99±2	100±2

* Average percent recovery ± standard deviation of triplicate analyses.

of the data shows this to be an artifact due to the high precision of the assay.

EXTRACTION STUDIES

Extraction studies were conducted to evaluate the extraction efficiencies of methylene chloride and 15% methylene chloride in hexane for PAH in water at pH 2, 7, and 10. Results of these analyses are shown in Table 7. ANOVA analysis of the extraction data indicates, as shown in Table 7, that methylene chloride is a significantly better extraction solvent for virtually all of the PAH. For several of the PAH, anthracene, pyrene, benz(b)fluoranthene, indeno(1,2,3,-cd)pyrene, and dibenz(a,h)anthracene, pH also had a significant effect, according to the ANOVA analysis. Inspection of the data indicates that in these cases pH 10 gave much lower recoveries than pH 7 or pH 2.

On the basis of these data it is apparent that methylene chloride is the extraction solvent of choice, and that while pH 10 can lead to lower recoveries, pH 7 and pH 2 generally give good recoveries.

PRESERVATION STUDIES

Preservation studies were conducted to evaluate the stability of PAH in water under selected conditions. These conditions include pH 2, 7, 10; 0 and 2 ppm chloride; and room temperature (RT) and 4°C.

Distilled water which had previously been buffered and/or chlorinated in accordance with the analytical matrix was spiked with PAH standard solution and stored for 7 days. Each storage condition was run in duplicate.

Following the 7 day storage each solution was extracted, concentrated to 1 ml using a Kuderna-Danish evaporator, and analyzed.

The results of these analyses which are corrected for extraction efficiencies are given in Table 8. These data have been presented in alternate format, along with the ANOVA results in Table 9.

The most curious results of these analyses is the zero percent recovery of naphthalene at RT, pH 7, 0 ppm Cl, and acenaphthylene at RT, pH 2, and 2 ppm Cl respectively. These analyses were repeated with fresh stock solution and stored for 7 days under identical conditions. Results were identical, i.e., 0 percent recovery for these two compounds. It is possible that chlorine is degrading the acenaphthylene in some way, but there seems to be no plausible explanation for the naphthylene results.

TABLE 7. EXTRACTION STUDIES

	15% $MeCl_2$/Hexane			$MeCl_2$			ANOVA Analysis	
	pH 2	pH 7	pH 10	pH 2	pH 7	pH 10	Solvent	pH
Naphthalene	66±8*	66±11	57±9	102±5	100±7	89±8	+	0
Acenaphthene	81±9	76±13	70±9	100±5	99±9	101±8	+	0
Anthracene	87±10	77±12	66±9	100±7	100±7	91±4	+	+
Pyrene	95±16	88±14	75±11	100±3	100±4	84±2	0	+
Chrysene	72±31	79±10	67±8	103±10	100±10	78±1	+	0
Benz(k)fluoranthene	63±25	64±18	59±7	104±14	101±5	63±4	+	+
Indeno(1,2,3-cd)pyrene	96±32	88±17	56±19	106±13	100±4	64±11	0	+
Dibenz(a,h)anthracene	64±20	60±10	41±11	97±11	99±2	59±6	+	+
Acenaphyhylene	91±9	99±14	93±14	81±2	71±2	69±1	+	0
Fluorene	97±8	107±22	110±8	91±0.5	80±2	79±3	+	0
Phenanthrene	106±15	103±25	119±12	90±2	88±3	88±3	+	0
Benz(a)anthracene	97±7	83±14	104±12	90±1	92±6	90±3	+	0
Benz(a)pyrene	72±6	74±16	91±24	86±2	101±7	89±7	+	0
Benz(ghi)perylene	57±13	61±22	67±2	89±2	91±6	88±4	+	0
Fluoranthene	102±11	97±17	129±29	89±1	89±4	91±1	0	0

* Average percent recovery ± standard deviation for triplicate or quadruplicate analyses.

+ Statistically significant differences at the 95% confidence level.

0 No significant difference at the 95% confidence level.

TABLE 8. PRESERVATION STUDIES

	RT 0 Cl	RT 2 Cl	4°C 0 Cl	4°C 2 Cl	RT 0 Cl	RT 2 Cl	4°C 0 Cl	4°C 2 Cl	RT 0 Cl	RT 2 Cl	4°C 0 Cl	4°C 2 Cl
Naphthalene	77±5 *	89±9	81±9	61±5	0	93±7	89±7	97±8	104±8	83±8	111±8	101±8
Acenaphthene	86±5	79±7	90±9	54±5	69±9	66±9	97±9	100±9	102±8	77±8	102±8	98±11
Anthracene	83±7	75±13	85±8	39±7	23±7	22±7	88±7	45±7	100±4	25±4	100±4	38±4
Pyrene	85±12	86±6	91±5	58±3	84±4	79±7	86±6	97±4	111±4	86±3	111±2	93±10
Chrysene	63±12	88±10	79±10	59±10	84±11	53±10	83±10	77±10	109±3	91±1	105±1	95±13
Benz(k)fluoranthene	80±14	91±14	80±14	54±14	69±7	82±5	73±5	65±6	125±4	105±4	121±6	103±4
Indeno(1,2,3-cd)pyrene	77±13	88±13	75±13	46±13	75±16	85±4	78±4	73±4	142±11	117±11	133±11	102±12
Dibenz(a,h)anthracene	73±11	88±11	75±11	61±11	74±9	78±16	70±2	60±8	124±18	88±6	105±6	90±8
Acenaphthylene	74±2	0	75±2	5±3	73±2	17±2	73±2	68±2	78±1	52±1	72±4	84±4
Fluorene	73±2	64±1	80±0.5	69±2	76±2	50±2	68±2	73±3	70±3	48±3	57±6	71±10
Phenanthrene	70±2	59±2	73±5	60±7	72±3	69±4	69±3	72±3	66±3	45±3	57±3	61±3
Fluoranthene	61±5	60±1	67±1	64±2	61±4	45±4	61±4	65±4	64±2	44±2	55±2	59±2
Benz(a)anthracene	61±1	9±1	74±1	10±1	73±12	58±6	65±6	64±6	74±3	50±5	61±3	63±3
Benz(a)pyrene	44±10	26±3	112±5	26±4	84±7	35±7	86±7	46±7	111±7	72±7	89±9	89±7
Benz(ghi)perylene	94±6	63±2	106±9	94±13	86±6	84±6	88±9	88±6	110±5	57±5	88±10	84±8
Average	73	64	78	50	67	61	78	73	95	69	91	82
	pH 2				pH 7				pH 10			

* Average recovery ± standard deviation for duplicate samples.

TABLE 9. PRESERVATION STUDIES DATA AND ANOVA ANALYSIS

Compound	Average Stability			ANOVA Analysis	Average Stability		ANOVA Analysis	Average Stability		ANOVA Analysis
	pH 2	pH 7	pH 10	pH	4°C	RT	TCMP	0 ppm	2 ppm	Chlorine
Naphthalene	77	70	100	+	90	61	+	77	87	+
Acenaphthene	77	83	95	+	90	67	+	91	79	+
Anthracene	71	45	57	+	66	55	+	79	41	+
Pyrene	80	87	100	0	89	89	0	94	83	+
Chrysene	72	54	100	0	83	81	0	87	77	+
Benzo(k)fluoranthene	76	72	108	+	83	92	+	91	83	+
Indeno(1,2,3-cd)pyrene	72	78	118	0	85	97	+	97	85	+
Dibenz(ah)anthracene	74	71	96	0	77	87	+	87	78	0
Acenaphthylene	39	58	72	+	64	49	+	74	38	+
Fluorene	72	67	50	+	70	63	0	70	55	0
Phenanthrene	66	71	57	0	65	63	0	68	61	+
Fluoranthene	63	58	55	+	62	55	+	62	56	+
Benz(a)anthracene	39	65	62	+	56	54	0	68	34	+
Benz(a)pyrene	52	63	90	+	75	62	+	88	49	+
Benz(ghi)perylene	89	86	85	0	91	68	+	95	78	+

+ Significantly differenct at 95% confidence level.

0 No significant difference at 95% confidence level.

From the data presented in Table 8, it is obvious that added chlorine reduces stability to some extent, although this is highly dependent on the particular compound and the pH level, as will be discussed later. In general, stability was lower at RT than at 4°C although for benzo(k)-fluoranthene, indeno(1,2,3-cd)pyrene, and dibenz(a,h)anthracene room temperature gave slightly better stability at RT. The effect of pH on stability varied from compound to compound, although normally stability increased in the order pH2 pH7 pH10. The effect on pH is difficult to interpret due to the fact that pH appears to alter the affect of added chlorine, which has been previously reported.[2] It appears that the effect of added chlorine is greatest at low pH. Three compounds, acenaphthalene, benz(a)anthracene, and benz(a)pyrene appear to be extremely unstable in the presence of added chlorine, especially at pH 2.

The data at pH 10 (Table 9) are complicated by the fact that, for several of the compounds, recoveries greater than 100% were indicated. The reason for the apparently high recoveries are not known, although improper instrument calibration seems the most likely problem.

On the basis of the data presented in Tables 8 and 9 we have concluded that 4°C, pH 7, and removal of chlorine is the optimum protocol for sample storage. Table 8 shows that this condition gives reasonably consistent stabilities (∿80%) for the various compounds. Although pH 10 gives somewhat better average recovery, it appears that this is a result of the artifactually high (>100%) recoveries for a few compounds, and that for the remaining compounds pH 7 and pH 10 give equivalent stabilities. The need for low temperature storage is ovbious from the data in Table 8 and is very critical for real world samples where bacterial growth must be prevented. The need for chlorine removal at pH 7 and 4°C is clear for a few of the compounds (e.g. benz(a)pyrene and anthracene) although for the remaining compounds its affect is negligible.

Using pH 7, and 4°C, and removal of chlorine these data indicate that stabilities of ∿80% can be expected for a seven-day storage period.

LC CLEAN UP STUDIES

The percent recoveries of the representative PAH from each LC separation scheme are listed in Table 10 below.

TABLE 10. PNA RECOVERY

	Silica				Alumina			
	% Recovery			Total	% Recovery			Total
Elution Volume*	10	20	30		10	20	30	
Naphthalene	<0.5	93.0	<0.5	93	100	<0.5	<0.5	100
Anthracene	0.5	104.5	<0.5	105	86	13.0	<0.5	99
Fluoranthene	0.5	96.5	<0.5	97	78	18.0	<0.5	96
Chrysene	1.0	97.0	<0.5	98	38	53.0	<0.5	91
Dibenz(a,h) anthracene	<0.5	92.0	<0.5	92	0.5	83.0	<0.5	83

* 40 percent methylene chloride in hexane.

From the above data, it is apparent that either LC separation scheme would be suitable as a clean up procedure. However, silica gel gave somewhat higher recoveries for the larger molecular weight components as well as exhibiting a considerably faster flow rate during the separation procedure. Thus, the use of silica gel was favored over alumina as the stationary phase for these clean up procedures. A 25 ml elution volume was considered to be optimum, based on these data.

WASTEWATER ANALYSIS

As stated in the Experimental Section, five water samples were selected for analysis using the method outlined. Analyses of the plastic industry wastewater and raw sewage were conducted using the Spherisorb ODS column while subsequent wastewaters were analyzed using the Perkin-Elmer HC-ODS column.

Initial wastewater analyses were conducted by HPLC using a Spherisorb ODS reverse phase column and selective fluorescent wavelength detection based on the work of Das and Thomas (12). Quantitation of benz(a)anthracene/chrysene using this method involves the use of the ratio of the response at λex 250 and λem >370 to the response at λex 280 and >389.

Analysis of the plastic industry wastewater indicated this source to have a large number of components as well as having a rather high fluorescent background level. A chromatogram illustrating this high background is shown in Figure 7. Triplicate injection of a single sample extract gave good repeatability; however, comparison of different aliquots from the same sample gave erratic peaks which made quantitation

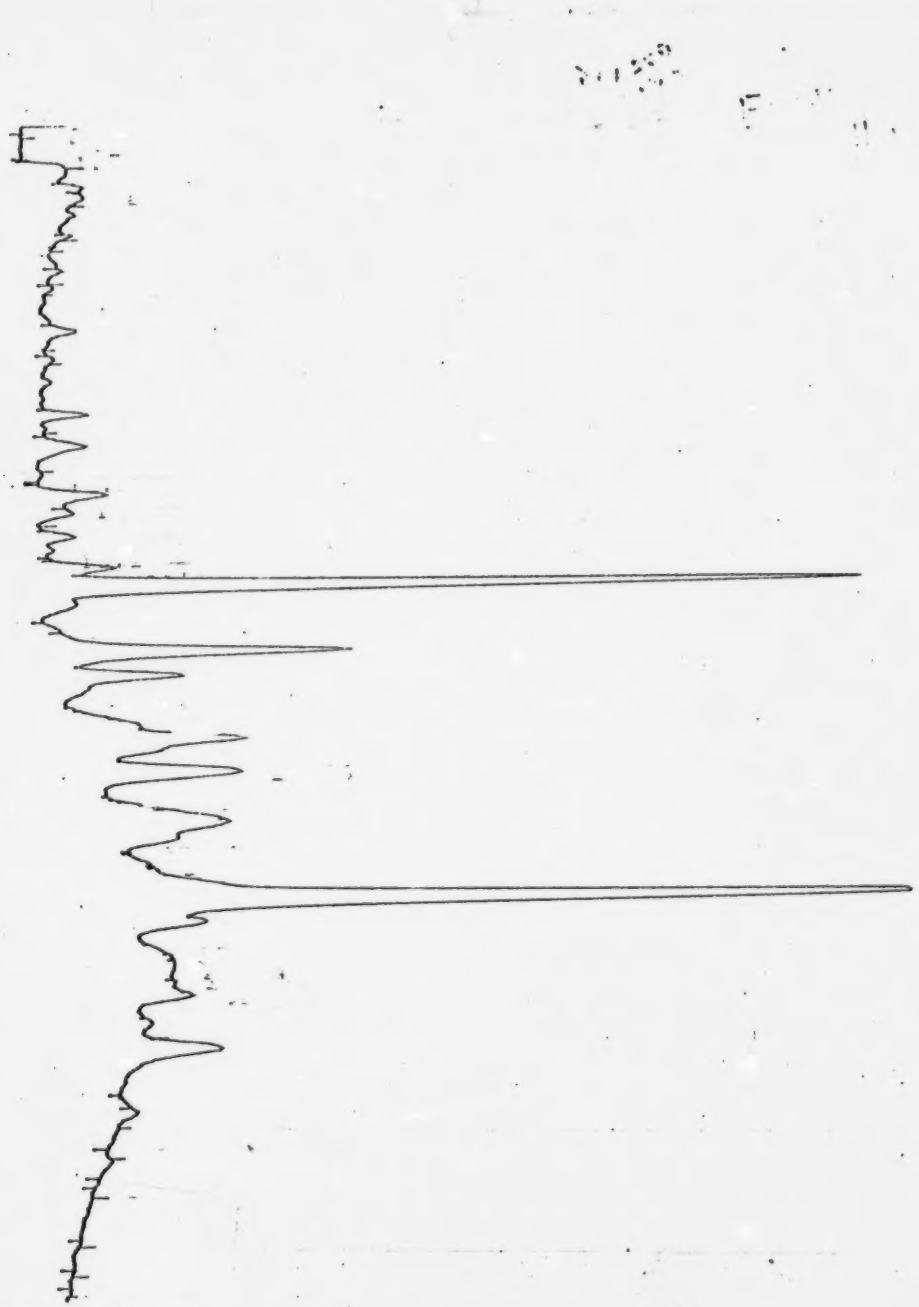

Figure 7. HPLC separation of water effluent extract from plastic industry (1st liter).

difficult. Retention times between spiked and unspiked samples agreed for some PAH species but the wavelength ratio λex 250 - λem 370/ λex 280 - λem 389 varied widely from the experimentally determined values. Examples of these variations are illustrated by comparison of Figure 7 with that of Figure 8. This variation occurred even among the spiked samples and the high recoveries observed are probably due to this wide variation in background PNA levels.

A possible explanation for these differences is that one aliquot may have contained more particulate material than another. Although the bulk sample bottle was shaken before removing the aliquot for extraction, there may have been differences in particulate content between aliquots. Since PAH is known to be distributed primarily onto the particulate, this is an important aspect in interpretation of the results.

Results of the plastic industry wastewater analysis are listed in Table 11. A chromatogram of a spiked sample is shown in Figure 9.

Subsequent wastewaters were homogenized prior to subdivision into 1 liter aliquots to eliminate these variations in background levels. However, the initial supply of plastic industry wastewater was exhausted and homogenization was not performed on this sample.

Raw municipal sewage from Columbus, Ohio, was obtained as the second wastewater to be studied. Initial extraction studies indicated a fluctuating background similar to that observed with the plastic industry effluent. However, homogenization of this sample produced a more consistent background on subsequent extractions. For real samples an alternate solution would be to collect a 1 liter sample, representative of a particular source, and extract the entire sample.

Analysis indicated the presence of chrysene at 2.4 ppb to be the PAH present in greatest concentration; BaP was present at 0.07 ppb. Results of these analyses are listed in Table 12. Typical chromatograms of spiked and unspiked sewage extract are shown in Figures 10 and 11. The detection limits (DL) for each sample were calculated based on the concentration required for a given compound to yield a peak two times greater than the baseline fluctuation (due to compounds eluting in the vicinity of the compound of interest) for the sample.

Treated municipal sewage water prior to chlorination was obtained as the third wastewater sample. Again homogenization was performed before aliquots were taken. Sample extracts indicted a lower and more uniform background than had been obtained with the raw sewage. Analysis indicated the presence of fewer PAH compounds than the untreated sewage as well as a lower concentration for chrysene at 0.3 ppb. However, an increase in the concentration of fluoranthene was detected from 0.5 ppb for the untreated sewage to 0.7 ppb for the treated.

Good PAH recoveries from spiked treated sewage were obtained except for naphthalene. These low results are due to the fact that the

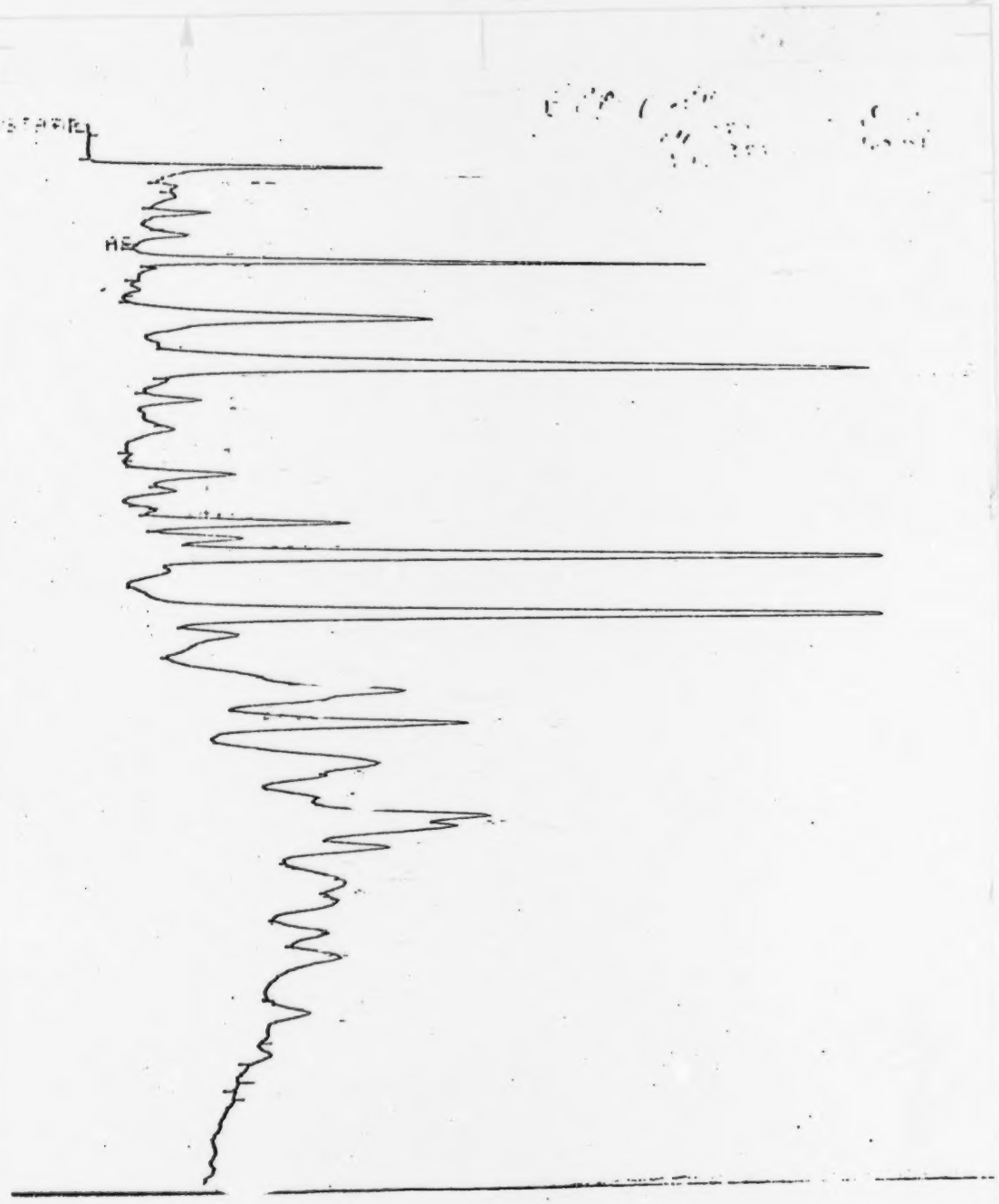

Figure 8. HPLC separation of water effluent extract from plastic industry (2nd liter).

TABLE 11. PLASTICS INDUSTRY WASTEWATER

Compound	Spiked	Spiked % recovery		Unspiked	
	Spiked level (ppb)	0 days	7 days	Background ppb	DL ppb
Naphthalene	70	76 ± 14	68 ± 19	<4.0	4.0
Acenaphthylene	450	134 ± 15	117 ± 13	<20	20.0
Acenaphthene	25	192 ± 28	181 ± 37	15.9 ± 5.3	0.8
Fluorene	23	152 ± 27	156 ± 27	0.7 ± 0.4	0.4
Phenanthrene	3.8	183 ± 16	179 ± 33	4.2 ± 2.3	2.1
Anthracene	7.9	181 ± 35	213 ± 48	7.8 ± 3.4	3.0
Fluoranthene	2.2	123 ± 23	182 ± 21	1.0 ± 0.06	0.23
Pyrene	6.9	223 ± 72	168 ± 25	4.8 ± 2.2	0.93
Benz(a)anthracene	0.66	208 ± 45	223 ± 31	0.45 ± 0.21	0.07
Chrysene	6.8	155 ± 26	169 ± 25	1.05 ± 0.30	0.1
Benz(b)fluoranthene	0.24	105 ± 13	135 ± 7	<0.03	0.03
Benz(k)fluoranthene	0.62	221 ± 24	156 ± 38	0.88 ± 0.30	0.03
Benz(a)pyrene	0.30	108 ± 12	122 ± 15	<0.03	0.03
Dibenz(a,h)anthracene	1.7	107 ± 13	115 ± 12	<0.06	0.06
Benz(ghi)perylene	3.4	127 ± 9	126 ± 18	<0.15	0.15
Indeno(1,2,3-cd)pyrene	1.4	133 ± 18	119 ± 13	<0.08	0.08

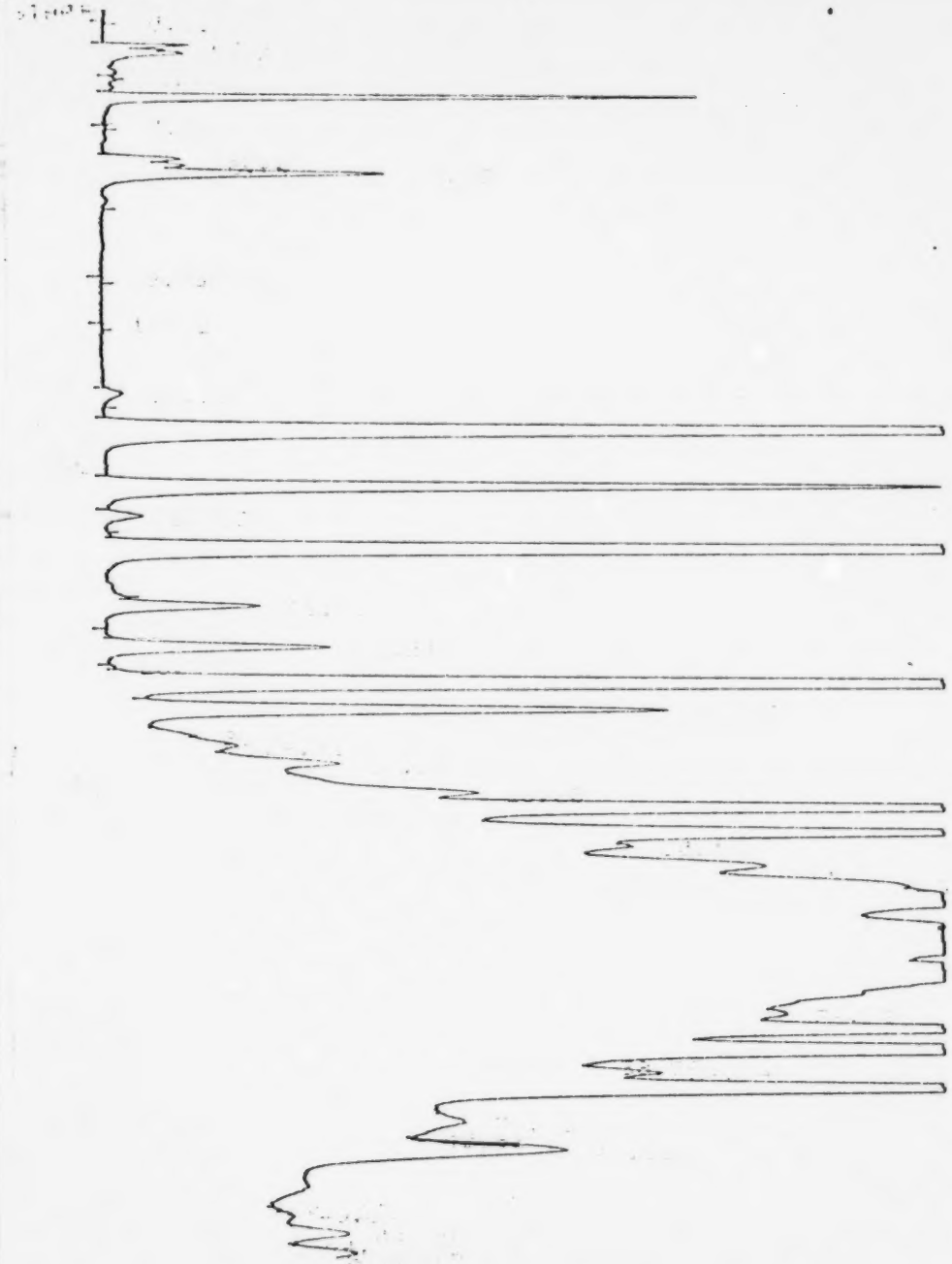

Figure 9. HPLC chromatogram of spiked water effluent extract from plastic industry.

TABLE 12. RAW SEWAGE WASTEWATER

Compound	Spiked			Unspiked	
	Spiked level (ppb)	% recovery 0 days	% recovery 7 days	Background ppb	MDL ppb
Naphthalene	70	108 ± 9	113 ± 7	<4	4.0
Acenaphthylene	450	119 ± 5	103 ± 5	<20	20
Acenaphthene	25	93 ± 4	85 ± 5	<0.8	0.8
Fluorene	23	101 ± 7	115 ± 13	<0.4	0.4
Phenanthrene	3.8	115 ± 8	129 ± 12	0.74 ± 0.15	0.3
Anthracene	7.9	114 ± 7	108 ± 7	0.3 ± 0.15	0.3
Fluoranthene	2.2	133 ± 12	134 ± 10	0.47 ± 0.14	0.02
Pyrene	6.9	127 ± 16	113 ± 12	1.0 ± 0.4	0.2
Benz(a)anthracene	0.66	102 ± 7	109 ± 9	0.19 ± 0.08	0.02
Chrysene	6.8	81 ± 3	93 ± 9	2.4 ± 1.05	0.2
Benz(b)fluoranthene	0.24	132 ± 24	133 ± 19	0.10 ± 0.04	0.02
Benz(k)fluoranthene	0.62	112 ± 15	116 ± 14	0.10 ± 0.04	0.02
Benz(a)pyrene	0.30	114 ± 9	90 ± 10	0.07 ± 0.01	0.03
Dibenz(a,h)anthracene	1.7	80 ± 6	89 ± 6	<0.06	0.06
Benz(ghi)perylene	3.4	81 ± 8	81 ± 12	<0.15	0.15
Indeno(1,2,3-cd)pyrene	1.4	98 ± 2	93 ± 7	<0.08	0.08

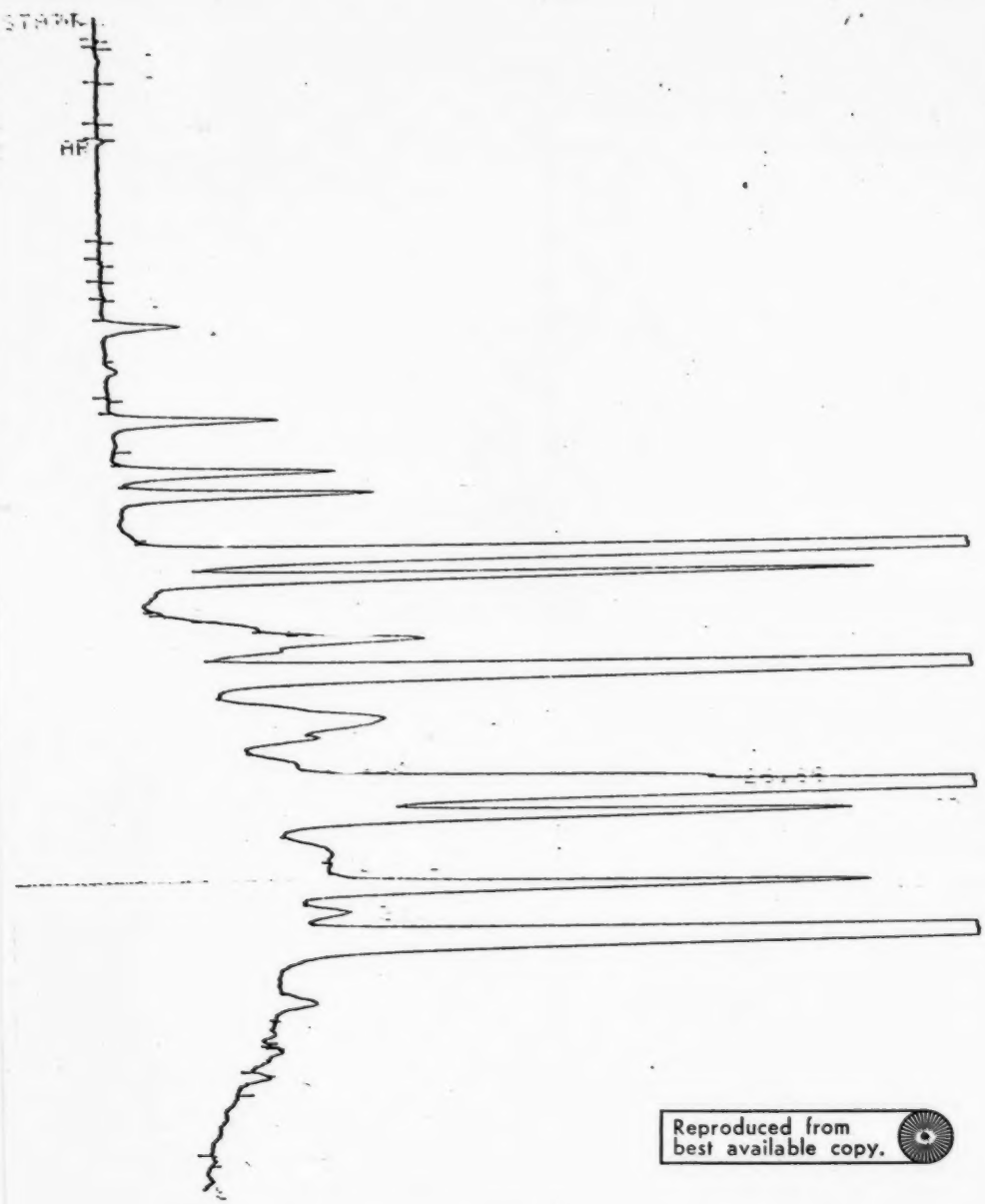

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Figure 10. HPLC chromatogram of spiked raw sewage.

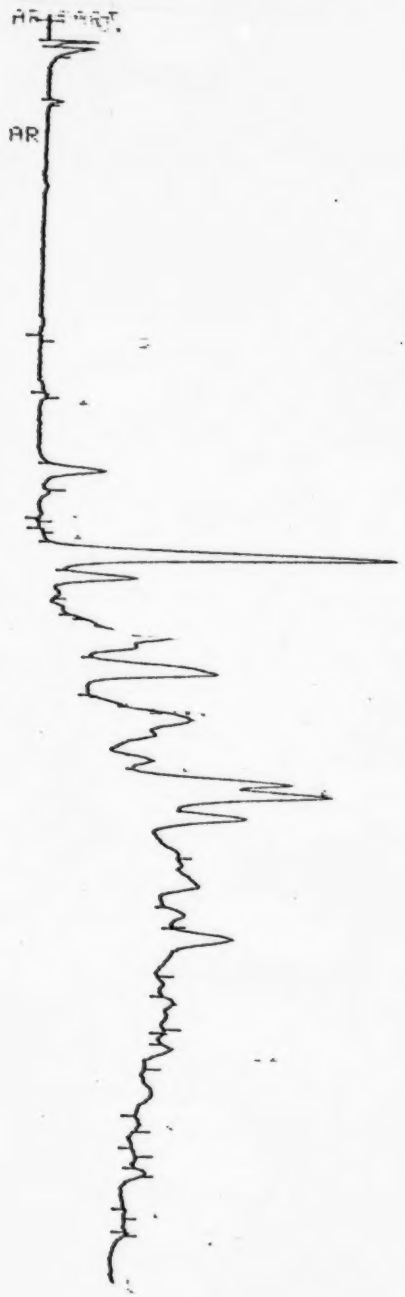

Figure 11. HPLC chromatogram of unspiked raw sewage.

concentration of naphthalene was close to the detection limit and the integrator did not integrate the sloping shoulders on this small peak. Since recoveries were calculated using peak area, a low result was obtained. Probably peak height would be a more accurate method for quantitation whenever the detection limit of the PAH compound is approached. Since our data was collected using a chromatographic data system, it is not possible to calculate recoveries on the basis of peak heights since many of the peaks were offscale on the attenuation setting used. Results of these analyses are listed in Table 13. Typical chromatograms of spiked and unspiked treated sewage are shown in Figures 12 and 13.

Flyash wash water from a coal fired power plant was collected as the fourth wastewater. The flyash which is collected by the electrostatic precipitators is received into large hoppers. These hoppers are emptied every few hours by slurrying the flyash with water which drains into a primary settling pond. The flyash wash samples were collected just at the outfall of the primary settling pond.

No difficulties resulted during the homogenization and subsequent analysis of this wastewater. Fluoranthene was the only PAH apparently present in this sample. As the water contact time with the flyash is relatively short, this is not surprising.

Results of these analyses are given in Table 14. Examples of spiked and unspiked chromatograms are shown in Figures 14 and 15.

An overflow secondary settling pond adjacent to the primary settling pond above was the source of the fifth wastewater. As the sample was collected in late summer, the water in both ponds was relatively low. Thus the primary settling pond water would not have overflowed into the secondary for quite some time. A surface grab sample of this water was obtained at the edge of the pond.

No difficulties resulted during the homogenization and subsequent analysis of this wastewater. Again, fluoranthene was the only PAH apparently present in this sample although at a slightly higher concentration than that found in the primary wash.

Results of these analyses are given in Table 15. Examples of spiked and unspiked chromatograms are shown in Figures 16 and 17.

The recovery data after seven days storage for each of the last four wastewater samples have been combined in Table 16 to give an average recovery for each of the PAH. The plastics industry sample data have not been included because of the problems encountered with sample homogeneity.

Based on the data obtained for the several wastèwater samples, it is obvious that the PAH were stable for seven days using the recommended preservation techniques and that the sample matrix did not seriously

TABLE 13. TREATED SEWAGE WASTEWATER

Compound	Spiked			Unspiked	
	Spiked level (ppb)	% recovery 0 days	% recovery 7 days	Background ppb	MDL ppb
Naphthalene	20	38 ± 3	24 ± 5	<4	4.0
Acenaphthylene	250	79 ± 8	88 ± 9	<20	20
Acenaphthene	11.6	79 ± 8	86 ± 7	1.8 ± 1.0	1.0
Fluorene	6.1	77 ± 5	75 ± 8	<0.4	0.4
Phenanthrene	5.0	83 ± 6	89 ± 9	<0.3	0.3
Anthracene	11.3	82 ± 6	88 ± 7	<0.3	0.3
Fluoranthene	0.3	127 ± 6	124 ± 7	0.7 ± 0.05	0.05
Pyrene	2.3	88 ± 4	89 ± 5	<0.2	0.2
Benz(a)anthracene	0.64	74 ± 6	71 ± 7	<0.02	0.02
Chrysene	2.0	89 ± 16	92 ± 9	0.3 ± 0.1	0.1
Benz(b)fluoranthene	0.3	88 ± 12	86 ± 9	<0.02	0.02
Benz(k)fluoranthene	0.14	86 ± 4	89 ± 7	<0.02	0.02
Benz(a)pyrene	0.21	101 ± 7	98 ± 8	<0.03	0.03
Dibenz(a,h)anthracene	0.4	86 ± 3	89 ± 5	<0.06	0.06
Benz(ghi)perylene	0.42	86 ± 6	90 ± 5	<0.15	0.15
Indeno(1,2,3-cd)pyrene	0.96	106 ± 6	97 ± 3	<0.08	0.08

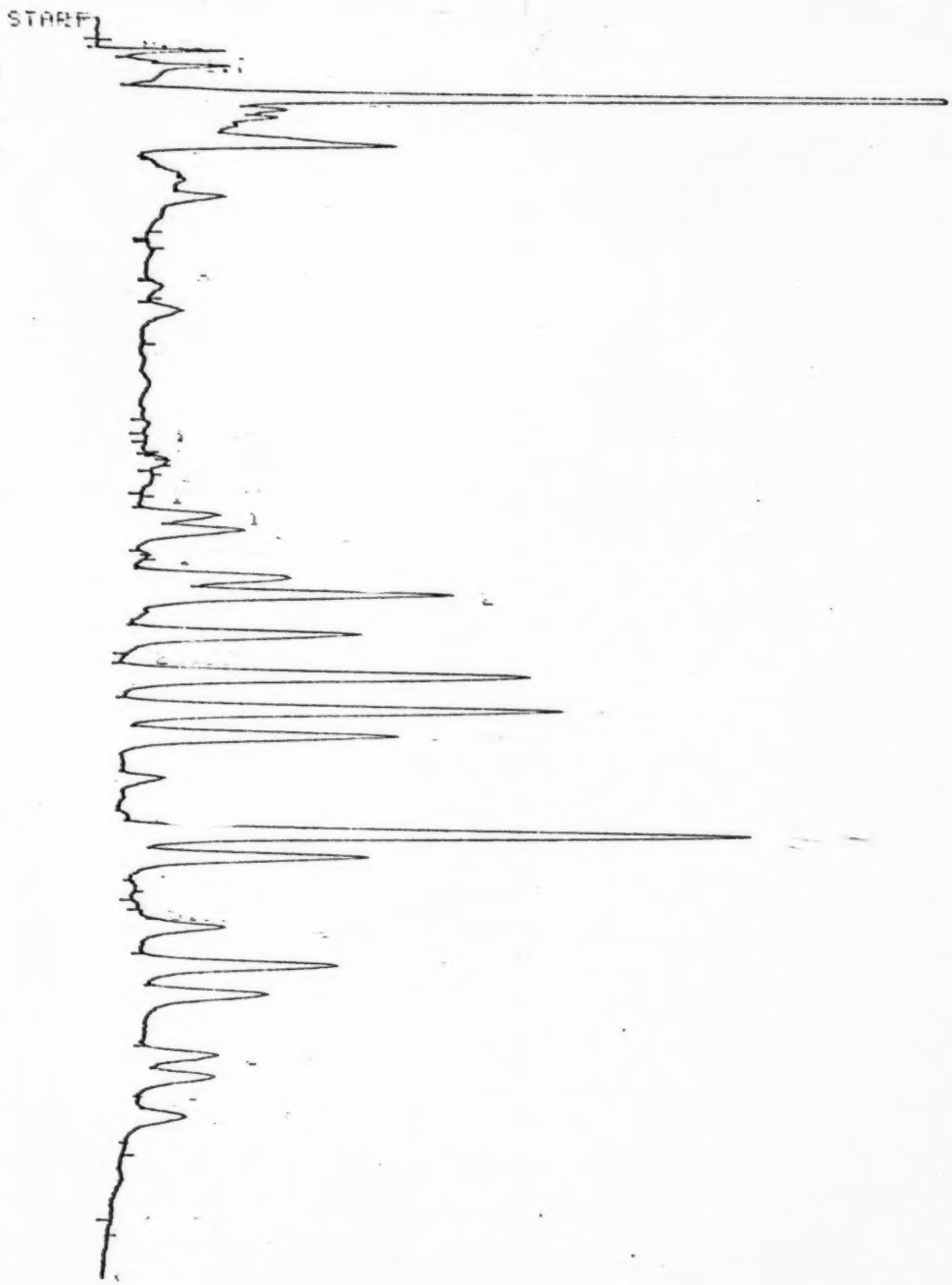

Figure 12. HPLC chromatogram of spiked treated sewage.

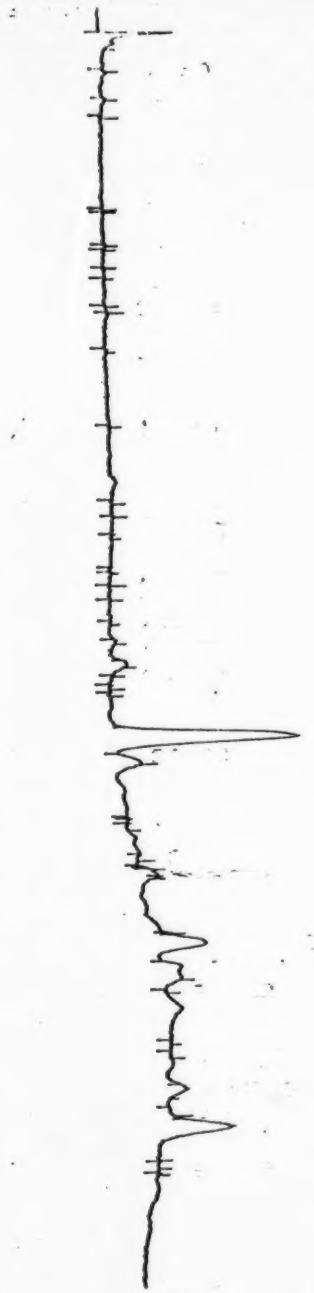

Figure 13. HPLC chromatogram of unspiked treated sewage.

TABLE 14. FLYASH WASH WASTEWATER

Compound	Spiked	Spiked % recovery		Unspiked	
	Spiked level (ppb)	0 days	7 days	Background ppb	MDL ppb
Naphthalene	40	87 ± 6	85 ± 6	<4	4
Acenaphthylene	250	88 ± 5	85 ± 7	<20	20
Acenaphthene	11.6	91 ± 4	87 ± 5	<0.8	0.8
Fluorene	6.1	86 ± 5	76 ± 7	<0.4	0.4
Phenanthrene	5.0	92 ± 8	94 ± 9	<0.24	0.24
Anthracene	11.3	92 ± 6	87 ± 6	<0.3	0.3
Fluoranthene	0.3	90 ± 6	89 ± 7	0.02 ± 0.01	0.01
Pyrene	2.3	94 ± 5	88 ± 6	<0.1	0.1
Benz(a)anthracene	0.64	91 ± 3	86 ± 10	<0.01	0.01
Chrysene	2.0	92 ± 7	86 ± 9	<0.1	0.1
Benz(b)fluoranthene	0.3	87 ± 7	80 ± 8	<0.01	0.01
Benz(k)fluoranthene	0.14	86 ± 6	86 ± 9	<0.01	0.01
Benz(a)pyrene	0.21	85 ± 5	87 ± 4	<0.01	0.01
Dibenz(a,h)anthracene	0.40	89 ± 7	85 ± 6	<0.02	0.02
Benz(ghi)perylene	0.42	89 ± 5	82 ± 8	<0.05	0.05
Indeno(1,2,3-cd)pyrene	0.96	85 ± 9	83 ± 7	<0.02	0.02

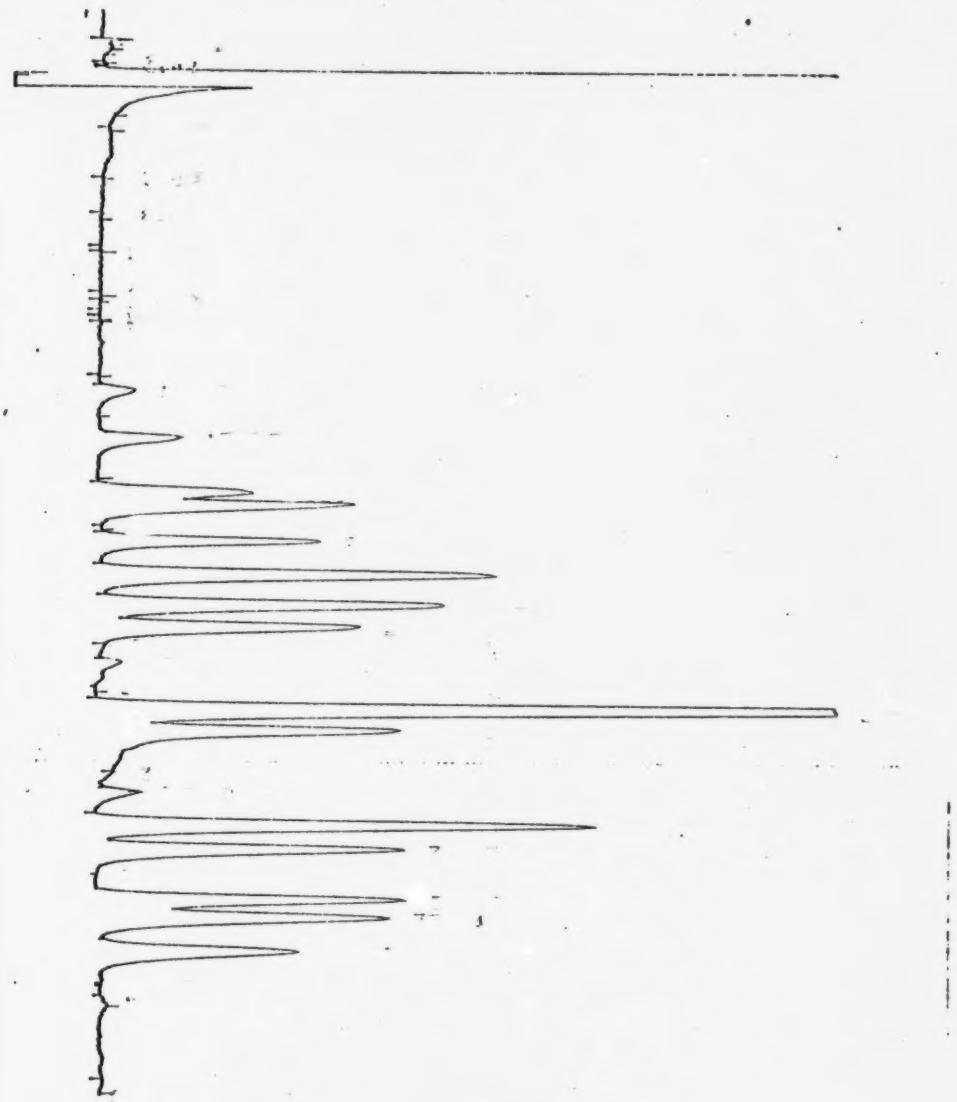

Figure 14. HPLC chromatogram of spiked flyash wash.

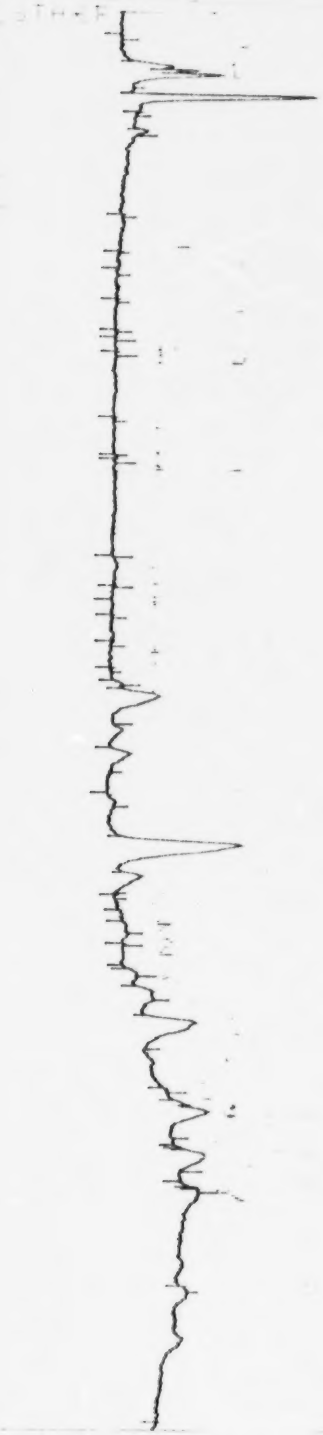

Figure 15. HPLC chromatogram of unspiked flyash wash.

TABLE 15. FLYASH SETTLING POND WASTEWATER

Compound	Spiked			Unspiked	
	Spiked level (ppb)	% recovery 0 days	% recovery 7 days	Background ppb	MDL ppb
Naphthalene	40	87 ± 17	83 ± 5	<4.0	4
Acenaphthylene	250	90 ± 5	89 ± 6	<20	20
Acenaphthene	11.6	96 ± 6	90 ± 5	<0.8	0.8
Fluorene	6.1	97 ± 8	93 ± 7	<0.4	0.4
Phenanthrene	5.0	93 ± 5	88 ± 8	<0.24	0.24
Anthracene	11.3	85 ± 5	86 ± 6	<0.03	0.03
Fluoranthene	0.3	115 ± 12	113 ± 14	0.03 ± 0.01	0.01
Pyrene	2.3	87 ± 6	86 ± 6	<0.1	0.1
Benz(a)anthracene	0.64	93 ± 4	87 ± 6	<0.01	0.01
Chrysene	2.0	86 ± 8	87 ± 5	<0.1	0.1
Benz(b)fluoranthene	0.3	88 ± 6	83 ± 5	<0.01	0.01
Benz(k)fluorantehen	0.14	90 ± 9	88 ± 6	<0.01	0.01
Benz(a)pyrene	0.21	92 ± 9	87 ± 5	<0.01	0.01
Dibenz(a,h)anthracene	0.40	90 ± 8	86 ± 4	<0.02	0.02
Benz(ghi)perylene	0.42	94 ± 6	85 ± 6	<0.05	0.05
Indeno(1,2,3-cd)pyrene	0.96	97 ± 8	89 ± 6	<0.02	0.02

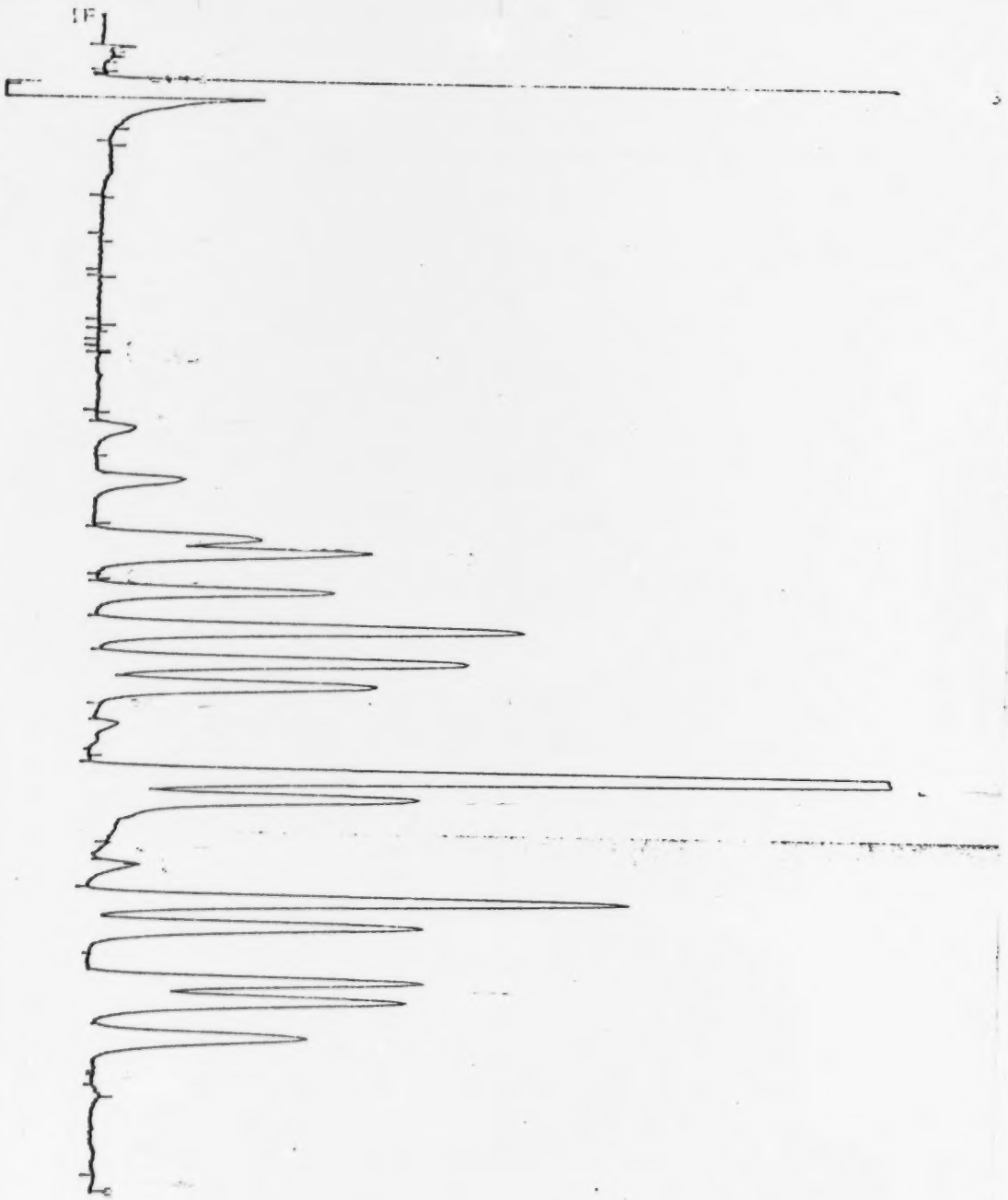

Figure 16. HPLC chromatogram of spiked flyash wash settling pond.

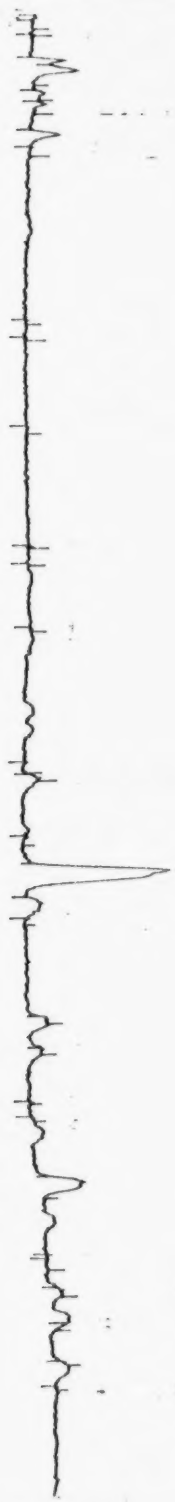

Figure 17. HPLC chromatogram of unspiked flyash wash settling pond.

TABLE 16. AVERAGE RECOVERIES FOR PAHs FROM WASTEWATER SAMPLES

Compound	Recovery
Napthalene	76±37*
Acenapthalene	91±8
Acenapthene	87±2
Fluorene	90±18
Phenanthrene	100±19
Anthracene	92±10
Fluoranthene	115±19
Pyrene	94±12
Benz(a)anthracene	88±15
Chrysene	90±4
Benz(b)fluoranthene	95±25
Benz(k)fluoranthene	94±14
Benz(a)pyrene	91±4
Dibenz(a,h)anthracene	87±2
Benz(ghi)perylene	85±4
Indeno(1,2,3-cd)pyrene	91±8

* Average recoveries for four wastewater samples after seven days storage ± the standard deviation of the four measurements.

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affect PAH recovery, since the recoveries for distilled water (e.g. preservation studies) and actual wastewater samples are virtually identical. The only serious problem encountered during the wastewater analysis was the problem of sample homogeneity, as discussed previously for the plastics industry sample. It appears that there are two alternative solutions to this problem: 1) thoroughly homogenize the sample before subsampling it or 2) obtain a representative sample from a particular source and analyze the entire sample.

SECTION 7

SUMMARY AND RECOMMENDATIONS

Several important points can be drawn from this analytical methods study for PAH in wastewater. These are:

- Sample homogenization is required in order to obtain a sample of uniform PAH content. Alternatively the entire sample can be extracted.

- For best preservation of PAH in water, the pH should be near 7, stored at near freezing temperatures and free chlorine should be removed by addition of a reducing agent.

- Methylene chloride is a suitable extraction solvent for PAH in water at pH 7 or pH 10.

- All 16 consent decree PAH can be separated by HPLC on a single stationary phase using a Perkin-Elmer HC-ODS column (2.6 x 250 mm I.D.).

- With proper wavelength selection, all consent decree PAH can be quantitated at sub-ppb levels.

- Although the silica LC clean up procedure probably removes the majority of the organics in the wastewater extract, a large number of components can still remain in the collected PAH fraction. This was observed in some of the wastewaters. Thus, there is a need for unambiguous peak identification. This could be achieved through the use of a stop-flow scanning spectrofluorimeter or by obtaining a UV or fluorescent spectra on collected fractions.

- PAH solutions are stable in DMSO and acetone for at least 90 days.

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APPENDIX A

POLYNUCLEAR AROMATIC HYDROCARBONS
METHOD 610

1. Scope and Application

1.1 This method covers the determination of certain polynuclear aromatic hydrocarbons (PAH). The following parameters may be determined by this method:

Parameter	STORET No.	CAS No.
Acenaphthene	34205	83-32-9
Acenaphthylene	34200	208-96-8
Anthracene	34220	120-12-7
Benzo(a)anthracene	34526	56-55-3
Benzo(a)pyrene	34247	50-32-8
Benzo(b)fluoranthene	34230	205-99-2
Benzo(ghi)perylene	34521	191-24-2
Benzo(k)fluoranthene	34242	207-08-9
Chrysene	34320	218-01-9
Dibenzo(a,h)anthracene	34556	53-70-3
Fluoranthene	34376	206-44-0
Fluorene	34381	86-73-7
Indeno(1,2,3-cd)pyrene	34403	193-39-5
Naphthalene	34696	91-20-3
Phenanthrene	34461	85-01-8
Pyrene	34469	129-00-0

1.2 This is a chromatographic method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. Method 625 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for many of the parameters listed in Section 1.1, using the extract produced by this method.

1.3 This method provides for both high performance liquid chromatographic (HPLC) and gas chromatographic (GC) approaches to the determination of PAHs. The gas chromatographic procedure does not adequately resolve the following four pairs of compounds:

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anthracene and phenanthrene; chrysene and benzo(a)anthracene; benzo(b)fluoranthene and benzo(k)fluoranthene; and dibenzo(a,h)-anthracene and indeno(1,2,3-cd)pyrene. Unless the purpose for the analysis can be served by reporting the sum of an unresolved pair, the liquid chromatographic approach must be used for these compounds. The liquid chromatographic method does resolve all 16 of the PAHs listed.

1.4 The method detection limit (MDL, defined in Section 15)[1] for each parameter is listed in Table 1. The MDL for a specific wastewater may differ depending upon the nature of interferences in the sample matrix.

1.5 The sample extraction and concentration steps in this method are essentially the same as in Methods 606, 608, 609, 611 and 612. Therefore, a single sample may be extracted to measure the parameters included in the scope of each of these methods, provided the concentration is high enough to permit selecting aliquots of the extract for cleanup, when required. Selection of the aliquots must be made prior to the solvent exchange steps of this method. The analyst is allowed the latitude, under Gas Chromatography (Section 13), to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters.

1.6 Any modification of this method, beyond those expressly permitted, shall be considered as major modifications subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.7 This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC and GC and in the interpretation of liquid and gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately 1-liter, is solvent extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and concentrated to a volume of 10 mL or less. The solvent is exchanged to cyclohexane prior to cleanup. Following cleanup, when using HPLC for determination of the PAHs, the solvent is exchanged to acetonitrile. Ultraviolet (UV) and fluorescence detectors are used with HPLC. When cleanup is not required and when FIDGC is used for determination, the methylene chloride extract may be analyzed directly. When cleanup is required, the cyclohexane exchange is made. Instrumental conditions are described which permit the separation and measurement of the PAH compounds[2].

2.2 A silica gel column cleanup procedure is provided to aid in the elimination of interferences that may be encountered.

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3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned[3]. Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and distilled water. It should then be drained dry, and heated in a muffle furnace at 400°C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional clean-up approaches to achieve the MDL listed in Table 1.

3.3 The extent of interferences that may be encountered using liquid chromatographic techniques has not been fully assessed. Although the HPLC conditions described allow for a unique resolution of the specific PAH compounds covered by this method, other PAH compounds may interfere.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method have not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets

should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified[4-6] for the information of the analyst.

4.2 The following parameters covered by this method have been tentatively classified as known or suspected, human or mammalian carcinogens; benzo(a)anthracene, benzo(a)pyrene and dibenzo(a,h)-anthracene.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber glass, 1-liter or 1-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only).

5.2.1 Separatory funnel - 2000-mL, with Teflon stopcock.

5.2.2 Drying column - Chromatographic column 400 mm long x 19 mm ID with coarse frit.

5.2.3 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.4 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.5 Snyder column, Kuderna-Danish - three-ball macro (Kontes

K-503000-0121 or equivalent).

5.2.6 Snyder column, Kuderna-Danish - two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.7 Vials - Amber glass, 10 to 15 mL capacity, with Teflon lined screw-cap.

5.2.8 Chromatographic column - 250 mm long x 10 mm ID with coarse fritted disc at bottom and Teflon stopcock.

5.3 Boiling chips - approximately 10/40 mesh. Heat to 400°C for 30 min. or Soxhlet extract with methylene chloride.

5.4 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

5.5 Balance - Analytical, capable of accurately weighing 0.0001g.

5.6 High performance liquid chromatographic apparatus (modular):

5.6.1 Gradient pumping system, constant flow.

5.6.2 Reverse phase column, 5 micron HC-ODS Sil-X, 250 mm x 2.6 mm ID (Perkin Elmer No. 089-0716 or equivalent).

5.6.3 Fluorescence detector, for excitation at 280 nm and emission greater than 389 nm cutoff (Corning 3-75 or equivalent). Fluorometers should have dispersive optics for excitation and can utilize either filter or dispersive optics at the emission detector.

5.6.4 UV detector, 254 nm, coupled to fluorescence detector.

5.6.5 Strip chart recorder compatible with detectors. Use of a data system for measuring peak areas and retention times is recommended.

5.7 Gas chromatograph - An analytical system complete with temperature programmable gas chromatograph suitable for on-column injection or splitless injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.7.1 Column - 1.8 m long x 2 mm ID pyrex glass packed with 3% OV-17 on Chromosorb W-AW-DCMS (100/120 mesh) or equivalent. This column was used to develop the retention time data in Table 2. Guidelines for the use of alternate column packings are provided in Section 13.

5.7.2 Detector - Flame ionization. This detector has proven effective in the analysis of wastewaters for the compounds

listed in the scope excluding the four pairs of unresolved compounds listed in Section 1.3. Guidelines for the use of alternate detectors are provided in Section 12.2.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Sodium thiosulfate - (ACS) Granular.

6.3 Cyclohexane, methanol, acetone, methylene chloride, and pentane - Pesticide quality or equivalent.

6.4 Acetonitrile, high purity water-HPLC quality, distilled in glass.

6.5 Sodium sulfate - (ACS) Granular, anhydrous. Purify by heating at 400°C for 4 hrs. in a shallow tray.

6.6 Silica gel - Grade 923 (100/200 mesh) dessicant (Davison Chemical or equivalent). Before use, activate for at least 16 hours at 130°C in a shallow glass tray, loosely covered with foil.

6.7 Stock standard solutions (1.00 µg/µL) - Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 grams of pure material. Dissolve the material in HPLC quality acetonitrile, dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.7.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Quality control check standards that can be used to determine the accuracy of calibration standards will be available from the U. S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, in Cincinnati, Ohic.

6.7.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicate a problem.

7. Calibration

7.1 Establish liquid or gas chromatographic operating parameters to produce resolution of the parameters equivalent to that indicated in Tables 1 or 2. The chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with acetonitrile. One of the external standards should be at a concentration near, but above, the method detection limit and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.2.2 Analyze each calibration standard (5 to 25 µL for HPLC and 2 to 5 µL for GC), and tabulate peak height or area responses against the mass injected. The results may be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (< 10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with acetonitrile. One of the standards should be at

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a concentration near, but above, the method detection limit and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.3.2 Analyze each calibration standard (5 to 25 µL for HPLC and 2 to 5 µL for GC) and tabulate peak height or area responses against concentration for each compound and internal standard, and calculate response factors (RF) for each compound using Equation 1.

$$\text{Eq. 1} \quad RF = (A_s C_{is})/(A_{is} C_s)$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard, (µg/L).
C_s = Concentration of the parameter to be measured, (g/L).

If the RF value over the working range is a constant (<10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.3.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. After January 1, 1982, ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate

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the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetronitrile 1000 times more concentrated than the selected concentrations. Quality control check sample concentrates, appropriate for use with this method, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the appropriate data from Table 3, determine the recovery and single operator precision expected for the method, and compare these results to the values calculated in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.

8.2.5 After January 1, 1982, the values for R and s must meet rigid method performance criteria provided by the U.S. EPA, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, before any samples may be analyzed.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance:

$$\text{Upper Control Limit (UCL)} = R + 3s$$
$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3.

The UCL and LCL can be used to construct control charts[7] that are useful in observing trends in performance. After January 1, 1982, the control limits above must be replaced by method performance criteria provided by the U.S. Environmental Protection Agency.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly[7].

8.4 The laboratory is required to collect a portion of their samples in duplicate to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 14.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a one-liter aliquot of reagent water, that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be

analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as chromatography with a dissimilar column or detector must be used. This may include the use of a mass spectrometer. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[8] should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon tubing and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction. PAHs are known to be light sensitive, therefore, samples, extracts and standards should be stored in amber or foil wrapped bottles in order to minimize photolytic decomposition. Fill the sample bottle and, if residual chlorine is present, add 80 mg of sodium thiosulfate per liter of sample. EPA Methods 330.4 and 330.5 may be used for measurement of residual chlorine[9]. Field test kits are available for this purpose.

9.3 All samples must be extracted within 7 days, and analysis completed within 40 days of extraction[2].

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel.

10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of ten minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.3 Add a second 60-mL volume of methylene chloride to the sample bottle, rinse and repeat the extraction procedure a second time,

combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the Kuderna-Danish if the requirements of Section 8.2 are met.

10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.6 Add 1 or 2 clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath (60 to 65°C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to Teflon-sealed screw-cap bottles and protected from light.

10.7 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%.

11.2 Before the silica gel cleanup technique can be utilized, the extract solvent must be exchanged to cyclohexane. Add a 1 to 10 mL aliquot of sample extract (in methylene chloride) and a boiling chip to a clean K-D concentrator tube. Add 4 mL cyclohexane and attach a micro-Snyder column. Prewet the micro-Snyder column by

adding 0.5 mL methylene chloride to the top. Place the micro-K-D apparatus on a boiling (100°C) water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete concentration in 5 to 10 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of the liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a minimum of cyclohexane. Adjust the extract volume to about 2 mL.

11.3 Silica gel column cleanup for PAHs.

11.3.1 Prepare a slurry of 10g activated silica gel in methylene chloride and place this in a 10-mm ID chromatography column. Gently tap the column to settle the silica gel and elute the methylene chloride. Add 1 to 2 cm of anhydrous sodium sulfate to the top of the silica gel.

11.3.2 Preelute the column with 40 mL of pentane. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, transfer the 2 mL of cyclohexane sample extract onto the column, using an additional 2 mL of cyclohexane to complete the transfer.

11.3.3 Just prior to exposure of the sodium sulfate layer to the air, add 25 mL pentane and continue elution of the column. Discard the pentane eluate.

11.3.4 Elute the column with 25 mL of methylene chloride/pentane (4 + 6)(V/V) and collect the eluate in a-500 mL K-D flask equipped with a 10-mL concentrator tube. Elution of the column should be at a rate of about 2 mL/min.

11.3.5 Concentrate the collected fraction to less than 10 mL by K-D techniques as in Section 10.6, using pentane to rinse the walls of the glassware. Proceed with HPLC or gas chromatographic analysis.

12. High Performance Liquid Chromatography (HPLC)

12.1 To the extract in the concentrator tube, add 4 mL of acetonitrile and a new boiling chip, then attach a micro-Snyder column. Increase the temperature of the hot water bath to 95 to 100°C. Concentrate the solvent as in Section 10. After cooling, remove the micro-Snyder column and rinse its lower joint into the concentrator tube with about 0.2 mL acetonitrile. Adjust the extract volume to 1.0 mL.

12.2 Table 1 summarizes the recommended HPLC column materials and

operating conditions for the instrument. This Table includes retention times, capacity factors, and method detection limits that were obtained under these conditions. The UV detector is recommended for the determination of naphthalene, acenaphthylene, acenapthene, and fluorene and the fluorescence detector is recommended for the remaining PAHs. Examples of the parameter separations achieved by this HPLC column are shown in Figures 1 and 2. Other HPLC columns, chromatographic conditions or detectors may be used if the requirements of Section 8.2 are met.

12.3 Calibrate the system daily as described in Section 7.

12.4 If the internal standard approach is being used, the internal standard must be added to sample extract and mixed thoroughly, immediately, before injection into the instrument.

12.5 Inject 5 to 25 µL of the sample extract using a high pressure syringe or a constant volume sample injection loop. Record the volume injected to the nearest 0.1 µL, and the resulting peak size in height or area units. Re-equilibrate the LC column at the initial gradient conditions for at least 10 minutes between injections.

12.6 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.7 If the peak height or area exceeds the linear range of the system, dilute the extract with acetonitrile and reanalyze.

12.8 If the peak area measurement is prevented by the presence of interferences, further cleanup is required.

13. Gas Chromatography

13.1 The packed column gas chromatographic procedure will not resolve certain isomeric pairs as indicated in Section 1.3 and Table 2. The liquid chromatographic procedure (Section 12) must be used for these materials. Capillary (open-tubular) columns may be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

13.2 To achieve maximum sensitivity with this method, the extract must be concentrated to 1.0 mL. Add a clean boiling chip to the methylene chloride extract in the concentrator tube. Attach a two-ball micro-Snyder column. Prewet the micro-Snyder column by adding about 0.5 mL of methylene chloride to the top. Place the micro K-D

apparatus on a hot water bath (60 to 65°C) so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation the balls will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus. Drain and cool for at least 10 minutes. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a small volume of methylene chloride. Adjust the final volume to 1.0 mL and stopper the concentrator tube.

13.3 Table 2 describes the recommended gas chromatographic column and operating conditions for the instrument. This Table includes retention times that were obtained under these conditions. An example of the parameter separations achieved by this column is shown in Figure 3. Other packed columns, chromatograhic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

13.4 Calibrate the gas chromatographic system daily as described in Section 7.

13.5 If the internal standard approach is being used, add the internal standard to sample extract and mix thoroughly, immediately, before injection into the instrument.

13.6 Inject 2 to 5 µL of the sample extract using the solvent-flush technique[10]. Smaller (1.0 µL) volumes may be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units.

13.7 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

13.8 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

13.9 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

14. Calculations

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14.1 Determine the concentration of individual parameters in the sample.

14.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated from Equation 2:

$$\text{Eq. 2.} \quad \text{Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.
V_i = Volume of extract injected (μL).
V_t = Volume of total extract (μL).
V_s = Volume of water extracted (mL).

14.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 and Equation 3.

$$\text{Eq. 3.} \quad \text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
C_{is} = Amount of internal standard added to each extract (μg).
V_o = Volume of water extracted, in liters.

14.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

14.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits established in Section 8.4, data for the affected parameters must be labeled as suspect.

15. Method Performance

15.1 Method detection limits - The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero[1]. The MDL concentrations listed in Table 1 were obtained using reagent water[11]. Similar results were achieved using representative wastewaters. MDL for the GC approach were not determined.

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15.2 This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from 8 x MDL to 800 x MDL[11], with the following exception: benzo(ghi)perylene recovery at 80 x and 800 x MDL were low (35% and 45% respectively).

15.3 In a single laboratory (Battelle Columbus Laboratories), using spiked wastewater samples, the average recoveries presented in Table 3 were obtained[2]. Each spiked sample was analyzed in triplicate on two separate days. The standard deviation of the precent recovery is also included in Table 3.

15.4 The Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

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TABLE 1

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
CONDITIONS AND METHOD DETECTION LIMITS

Parameter	Retention Time (min)	Capacity Factor (k')	Method Detection Limit (µg/L)[a]
Naphthalene	16.6	12.2	1.8
Acenaphthylene	18.5	13.7	2.3
Acenaphthene	20.5	15.2	1.8
Fluorene	21.2	15.8	0.21
Phenanthrene	22.1	16.6	0.64
Anthracene	23.4	17.6	0.66
Fluoranthene	24.5	18.5	0.21
Pyrene	25.4	19.1	0.27
Benzo(a)anthracene	28.5	21.6	0.013
Chrysene	29.3	22.2	0.15
Benzo(b)fluoranthene	31.6	24.0	0.018
Benzo(k)fluoranthene	32.9	25.1	0.017
Benzo(a)pyrene	33.9	25.9	0.023
Dibenzo(a,h)anthracene	35.7	27.4	0.030
Benzo(ghi)perylene	36.3	27.8	0.076
Indeno(1,2,3-cd)pyrene	37.4	28.7	0.043

HPLC conditions: Reverse phase HC-ODS Sil-X 2.6 mm x 250 mm Perkin-Elmer column; isocratic elution for 5 min. using acetonitrile/water (4 + 6), then linear gradient elution to 100% acetonitrile over 25 minutes; flow rate is 0.5 mL/min. If columns having other internal diameters are used, the flow rate should be adjusted to maintain a linear velocity of 2 mm/sec.

[a]The method detection limit for naphthalene, acenaphthylene, acenaphthene, and fluorene were determined using a UV detector. All others were determined using a fluorescence detector.

TABLE 2

GAS CHROMATOGRAPHIC OPERATING CONDITIONS
AND RETENTION TIMES

Parameter	Retention Time (min)
Naphthalene	4.5
Acenaphthylene	10.4
Acenaphthene	10.8
Fluorene	12.6
Phenanthrene	15.9
Anthracene	15.9
Fluoranthene	19.8
Pyrene	20.6
Benzo(a)anthracene	20.6
Chrysene	24.7
Benzo(b)fluoranthene	28.0
Benzo(k)fluoranthene	28.0
Benzo(a)pyrene	29.4
Dibenzo(a,h)anthracene	36.2
Indeno(1,2,3-cd)pyrene	36.2
Benzo(ghi)perylene	38.6

GC conditions: Chromosorb W-AW-DCMS (100/120 mesh) coated with 3% OV-17, packed in a 6' x 2 mm ID glass column, with nitrogen carrier gas at a flow rate of 40 mL/min. Column temperature was held at 100°C for 4 minutes, then programmed at 8°/minute to a final hold at 280°C.

TABLE 3

SINGLE OPERATOR ACCURACY AND PRECISION

Parameter	Average Percent Recovery	Standard Deviation %	Spike Range (µg/L)	Number of Analyses	Matrix Types
Acenaphthene	88	5.7	11.6-25	24	4
Acenaphthylene	93	6.4	250-450	24	4
Anthracene	93	6.3	7.9-11.3	24	4
Benzo(a)anthracene	89	6.9	0.64-0.66	24	4
Benzo(a)pyrene	94	7.4	0.21-0.30	24	4
Benzo(b)fluoranthene	97	12.9	0.24-0.30	24	4
Benzo(ghi)perylene	86	7.3	0.42-3.4	24	4
Benzo(k)fluoranthene	94	9.5	0.14-6.2	24	4
Chrysene	88	9.0	2.0-6.8	24	4
Dibenzo(a,h)anthracene	87	5.8	0.4-1.7	24	4
Fluoranthene	116.	9.7	0.3-2.2	24	4
Fluorene	90.	7.9	6.1-23	24	4
Indeno(1,2,3-cd)pyrene	94	6.4	0.96-1.4	24	4
Naphthalene	78	8.3	20-70	24	4
Phenanthrene	98	8.4	3.8-5.0	24	4
Pyrene	96	8.5	2.3-6.9	24	4